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PROCEEDINGS OF THE FIFTY-SECOND ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1936

The fifty-second annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., November 29, December 1 and 2, 1936.

The meeting was called to order by the president, H. H. Hanson, State Board of Agriculture, Dover, Del., on the morning of November 29, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER, 1937

President

C. C. McDonnell, U. S. Food and Drug Administration, Washington, D. C.

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H. R. Kraybill, Purdue University, Lafayette, Ind.

Secretary-Treasurer

W. W. Skinner, U. S. Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee

W. S. Frisbie, Washington, D. C.
C. L. Hare, Auburn, Ala.
L. B. Broughton, College Park, Md.
H. H. Hanson, Dover, Del.

Permanent Committees

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

H. A. Lepper (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*

Subcommittee A: G. L. Bidwell (1938), (U. S. Food and Drug Adm., Washington, D. C.), *Chairman;* G. E. Grattan (1940) and H. A. Halvorson (1942). [Standard solutions; insecticides, fungicides, and caustic poisons (fluorine compounds, pyrethrins, derris, and cubé, naphthalene in poultry lice products); soils and liming materials (hydrogen-ion concentration—soils of arid and semi-arid regions and soils of humid regions, liming materials, less common metals in soils, fluorine, selenium); feeding stuffs (fluorine, stock feed adulteration, mineral mixed feeds, moisture, lactose in mixed feeds, fat in fish meal, hydrocyanic acid in glucoside-bearing materials, biological methods for determination of vitamin D carriers, biological methods for vitamin B complexes, technic and details of biological methods, vitamin D carriers, carotene, qualitative tests for proteins); fertilizers (phosphoric acid, nitrogen, magnesium and manganese

acid and base-forming quality, potash); plants (less common metals, total chlorine, carbohydrates, inulin, hydrocyanic acid, forms of nitrogen, sodium and potassium); lignin, enzymes (pepsin, papain), paints, paint materials and varnishes (accelerated testing of paints); vitamins (vitamin A, vitamin D); leather and tanning materials, disinfectants.]

SUBCOMMITTEE B: L. B. Broughton (1938), (University of Maryland, College Park, Md.), *Chairman;* H. J. Fisher (1940) and A. E. Paul (1942). [Naval stores (rosin, turpentine); radioactivity (quantum counter, gamma ray), drugs (microchemical methods for alkaloids, microchemical methods for synthetics, hypophosphites, santonin, phenolphthalein and calomel in tablets; guaiacol, rhubarb and rhaponticum, hexylresorcinol, ergot alkaloids, nitroglycerin in mixtures, iodine ointment, biological testing, acetphenetidin in presence of caffeine and aspirin, pyridium, gums, cinchophen in presence of salicylates, theobromine-calcium tablets, chlorbutanol, aspirin and phenolphthalein mixtures, homatropine tablets, cubeb, emulsion of cod liver oil, elixir of terpin hydrate and codeine, aminopyrine and phenobarbital in mixtures, daphnia methods, ointment of mercuric nitrate, effervescent potassium bromide with caffeine and similar preparations, cosmetics.]

SUBCOMMITTEE C: J. O. Clarke (1938), (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* G. G. Frary (1940) and W. B. White (1942). [Dairy products (butter—preparation of sample and fat, cheese, malted milk—chemical methods, microanalytical methods, dried milk, milk proteins, lactose in milk, gelatin, extraneous matter, decomposition, neutralizers); oils, fats, and waxes (refractometric determination of oil in seeds, free fatty acids, thiocyanogen); eggs and egg products (unsaponifiable constituents and fat, detection of decomposition; glycerol, sugar, and added salt, dried eggs); metals in foods (arsenic, copper, zinc, fluorine, lead, mercury, selenium); canned foods (tomato products), vinegars (ash), meats and meat products, gums in foods, spices, microbiological methods (canned vegetables, canned tomatoes and fruits, canned fish products, canned meats, sugar); fish and other marine products.]

SUBCOMMITTEE D: J. A. LeClerc (1938), (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;* W. C. Jones (1940) and J. W. Sale (1942). Sugars and sugar products (acetyl-methyl carbinol and diacetyl in food products, unfermentable sugars in molasses, honey, refractive indices of sugar solutions, maple products; drying, densimetric, and refractometric methods; polariscopic methods, chemical methods for reducing sugars, lead precipitate); waters, brine, and salt (mineral salts and effervescent salts); alcoholic beverages (malt beverages, extracts, and sirups, and brewing materials; diastatic and proteolytic activity of malt, CO_2 in beer, malt extract in malt, malt adjuncts, wines, alchol by use of the ebullioscope, volatile acidity, SO_2 in wines and beers, detection of adulteration of distilled spirits, cordials and liqueurs); food preservatives (saccharin, benzoate of soda); coloring matters in foods, fruits and fruit products (soluble solids and effect of acids on sugar on drying, electrolytic titration acidity, fruit acids, polariscopic methods for jams, jellies, and preserves); flavors and non-alcoholic beverages, cacao products, baking powders and baking chemicals (fluorine, lead); cereal foods (soya flour in foods, macaroni, whole wheat flour, phosphated flour, ash in flour, macaroni products, and baked products; H-ion concentration of flour, starch in flour, flour-bleaching chemicals, CO_2 in self-rising flour; milk solids in milk bread, viscosity of flour, cold water extract flour, ergot in flour, catalase and proteolytic enzymes, color in flour); microchemical methods.

Committee to Cooperate with Other Committees on Food Definitions

C. D. Howard (Board of Health, Concord, N. H.), *Chairman*
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Representatives on the Board of Governors of the Crop Protection Institute of the National Research Council

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Referees and Associate Referees

STANDARD SOLUTIONS:

General referee: R. L. Vandaveer, Food and Drug Adm., Chicago, Ill.

INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS:

General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

PYRETHRINS, DERRIS AND CUBÉ:

Associate referee: J. J. T. Graham.

FLUORINE COMPOUNDS:

Associate referee: R. H. Carter, Bureau of Entomology and Plant Quarantine, Washington, D. C.

NAPHTHALENE IN POULTRY LICE PRODUCTS:

Associate referee: Roswell Jinkins, Food and Drug Adm., Chicago, Ill.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

HYDROGEN-ION CONCENTRATION:

a. SOILS OF ARID AND SEMI-ARID REGIONS:

Associate referee: W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

b. SOILS OF HUMID REGIONS:

Associate referee: J. B. Hester, Virginia Truck Experiment Sta., Norfolk, Va.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

SELENIUM IN SOILS:

Associate referee: K. T. Williams, Bureau of Chemistry and Soils, Washington, D. C.

FEEDING STUFFS:

General referee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

FLUORINE:

Associate referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

STOCK FEED ADULTERATION:

Associate referee: P. B. Curtis, Agricultural Experiment Station, Lafayette Ind.

MINERAL MIXED FEEDS:

Associate referee: H. E. Perkins, Manhattan, Kans.

LACTOSE IN MIXED FEEDS:

Associate referee: D. A. Magraw, Amer. Dry Milk Inst., Chicago, Ill.

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS:

Associate referee: R. A. Green, University of Arizona, Tucson, Ariz.

BIOLOGICAL METHODS FOR DETERMINATION OF VITAMIN D CARRIERS

Associate referee: W. B. Griem, Department of Agriculture and Markets, Madison, Wis.

BIOLOGICAL METHODS FOR VITAMIN B COMPLEXES:

Associate referee: C. A. Elvehjem, Department of Agricultural Chemistry, Madison, Wis.

TECHNIC AND DETAILS OF BIOLOGICAL METHODS, VITAMIN D CARRIERS:

Associate referee: L. L. Latchat, Dept. of Agriculture, Dairy and Food, St. Paul, Minn.

CAROTENE

Associate referee: V. E. Munsey, Food and Drug Adm., Washington, D.C.,

QUALITATIVE TESTS FOR PROTEINS:

Associate referee: D. Breese Jones, Bureau of Chemistry and Soils, Washington, D. C.

FAT IN FISH MEAL:

Associate referee: R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

FERTILIZERS:

General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:

Associate referee. W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN

Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

MAGNESIUM AND MANGANESE:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH:

Associate referee: O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

ACID AND BASE-FORMING QUALITY:

Associate referee: L. E. Horat, Agricultural Experiment Station, Lafayette, Ind.

PLANTS:

General referee: O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

LESS COMMON METALS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE:

Associate referee: H. L. Wilkins, Bureau of Plant Industry, Washington, D. C.

CARBOHYDRATES:

Associate referee: J. T. Sullivan, Agricultural Experiment Station, Lafayette, Ind.

INULIN:

Associate referee: T. G. Phillips, University of New Hampshire, Durham, N. H.

FORMS OF NITROGEN:

Associate referee: H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

HYDROCYANIC ACID IN PLANTS:

Associate referee: R. A. Green, University of Arizona, Tucson, Ariz.

SODIUM AND POTASSIUM:

Associate referee: R. T. Milner, Regional Soybean Ind. Prods. Lab., Urbana, Ill.

LIGNIN:

General referee: M. Phillips, Bureau of Chemistry and Soils, Washington, D. C.

ENZYMES:

General referee: A. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

PEPSIN:

Associate referee: A. K. Balls.

PAPAIN:

Associate referee: T. L. Swenson, Bureau of Chemistry and Soils, Washington, D. C.

PAINTS, PAINT MATERIALS AND VARNISHES:

General referee: C. S. Ladd, Food Commissioner and Chemist, Bismarck, N. D.

ACCELERATING TESTING OF PAINTS:

Associate referee: L. L. Carrick, Agricultural Experiment Station, Fargo, N. D.

VITAMINS:

General referee: E. M. Nelson, Food and Drug Adm., Washington, D. C.

VITAMIN A:

Associate referee: J. B. Wilkie, Food and Drug Adm., Washington, D. C.

VITAMIN D:

Associate referee: W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

LEATHERS AND TANNING MATERIALS:

General referee: I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

DISINFECTANTS:

General referee: C. N. Brewer, Food and Drug Adm., Washington, D. C.

NAVAL STORES:

General referee: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

ROSIN:

Associate referee: F. P. Veitch.

TURPENTINE:

Associate referee: V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

RADIOACTIVITY:

General referee: C. H. Badger, Food and Drug Adm., Washington, D. C.

QUANTUM COUNTER:

Associate referee: J. A. Mathews, Food and Drug Adm., Washington, D. C.

GAMMA RAY SCOPE:

Associate referee: C. H. Badger.

DRUGS:

General referee: L. E. Warren, Food and Drug Adm., Washington, D. C.

ACETPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN:

Associate referee: S. M. Berman, Food and Drug Adm., Philadelphia, Pa.

AMINOPYRINE AND PHENOBARBITAL IN MIXTURES:

Associate referee: E. C. Payne, Food and Drug Adm., Chicago, Ill.

ASPIRIN AND PHENOLPHTHALEIN MIXTURES:

Associate referee: G. M. Johnson, Food and Drug Adm., Minneapolis, Minn.

BIOLOGICAL TESTING:

Associate referee: W. T. McClosky, Food and Drug Adm., Washington, D. C.

CHLORBUTANOL:

Associate referee: F. C. Sinton, Food and Drug Adm., New York City.

CINCHOPHEN IN PRESENCE OF SALICYLATES:

Associate referee: A. I. Cohen, Food and Drug Adm., Chicago, Ill.

CUBEB:

Associate referee: J. F. Clevenger, Food and Drug Adm., New York City.

DAPHNIA METHODS:

Associate referee: A. Viehoever, Philadelphia, Pa.

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE:

Associate referee: H. G. Underwood, Food and Drug Adm., Cincinnati, Ohio.

ELIXIR OF TERPIN HYDRATE AND CODEINE:

Associate referee: Jonas Carroll, Food and Drug Adm., Cincinnati, O.

EMULSIONS OF COD LIVER OIL:

Associate referee: W. F. Kunke, Food and Drug Adm., Chicago, Ill.

ERGOT ALKALOIDS:

Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

GUAIACOL:

Associate referee: K. L. Milstead, Food and Drug Adm., Chicago, Ill.

GUMS:

Associate referee: J. H. Cannon, Food and Drug Adm., Chicago, Ill.

HEXYLRESORCINOL:

Associate referee: M. L. Yakovitz, Food and Drug Adm., San Francisco, Calif.

HOMATROPINE TABLETS:

Associate referee: E. M. Hoshall, Food and Drug Adm., Baltimore, Md.

HYPOPHOSPHITES:

Associate referee: H. R. Bond, Food and Drug Adm., Chicago, Ill.

IODINE OINTMENT:

Associate referee: W. F. Reindollar, State Dept. of Health, Baltimore, Md.

MICROCHEMICAL METHODS FOR ALKALOIDS:

Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR SYNTHETICS:

Associate referee: I. S. Shupe, Food and Drug Adm., St. Louis, Mo.

NITROGLYCERINE IN MIXTURES:

Associate referee: O. A. Kenworthy, Food and Drug Adm., New York City.

OINTMENT OF MERCURIC NITRATE:

Associate referee: H. O. Moraw, Food and Drug Adm., Chicago, Ill.

PYRIDIUM:

Associate referee: H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

RHUBARB AND RHAPONTICUM:

Associate referee: E. H. Wirth, University of Illinois, Chicago, Ill.

SANTONIN, PHENOLPHTHALEIN AND CALOMEL IN TABLETS:

Associate referee: H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

THEOBROMINE-CALCIUM TABLETS:

Associate referee: P. S. Jorgensen, Food and Drug Adm., San Francisco, Calif.

THEOPHYLLINE SODIUM SALICYLATE:

Associate referee: M. Harris, Food and Drug Adm., Chicago, Ill.

COSMETICS:

General referee: C. D. Howard, State Health Dept., Concord, N. H.

DAIRY PRODUCTS:

General referee: G. G. Frary, Dairy and Food Dept., Vermillion, S. D.

BUTTER—PREPARATION OF SAMPLE AND FAT:

Associate referee: R. S. Pruitt, Food and Drug Adm., New Orleans, La.

CHEESE:

Associate referee: C. B. Stone, Food and Drug Adm., Cincinnati, Ohio.

MALTED MILK, CHEMICAL METHODS:

Associate referee: F. Hillig, Food and Drug Adm., Washington, D. C.

MALTED MILK, MICROANALYTICAL METHODS:

Associate referee: G. L. Keenan, Food and Drug Adm., Washington, D. C.

DRIED MILK:

Associate referee: F. Hillig.

MILK PROTEINS:

Associate referee: W. E. Peterson, College of Agriculture, St. Paul, Minn.

GELATIN IN DAIRY PRODUCTS:

Associate referee: G. A. Richardson, University of California, Davis, Calif.

LACTOSE IN MILK:

Associate referee: E. R. Garrison, University of Missouri, Columbia, Mo.

EXTRANEOUS MATTER IN DAIRY PRODUCTS:

Associate referee: J. D. Wildman, Food and Drug Adm., Washington, D. C.

DECOMPOSITION IN DAIRY PRODUCTS:

Associate referee: J. D. Clarke, Food and Drug Adm., Chicago, Ill.

NEUTRALIZERS IN DAIRY PRODUCTS:

Associate referee: F. Hillig, Food and Drug Adm., Washington, D. C.

OILS, FATS AND WAXES:

General referee: G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:

Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

THIOCYANOGEN NUMBER:

Associate referee: G. S. Jamieson.

FREE FATTY ACIDS:

Associate referee: R. S. McKinney, Bureau of Chemistry and Soils, Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: H. A. Lepper, Food and Drug Adm., Washington, D. C.

UNSAPONIFIABLE CONSTITUENTS AND FAT:

Associate referee: E. O. Haenni, Food and Drug Adm., Washington, D. C.

GLYCEROL, SUGAR, AND ADDED SALT:

Associate referee: L. C. Mitchell, Food and Drug Adm., Minneapolis, Minn.

DETECTION OF DECOMPOSITION:

Associate referee: J. Callaway, Jr., Food and Drug Adm., New York City.

DRIED EGGS:

Associate referee: F. J. McNall, Food and Drug Adm., Chicago, Ill.

METALS IN FOODS:

General referee: H. J. Wichmann, Food and Drug Adm., Washington, D. C.

ARSENIC:

Associate referee: C. C. Cassil, Bureau of Entomology and Plant Quarantine, Washington, D. C.

COPPER:

Associate referee: D. L. Drabkin, University of Pennsylvania, Philadelphia, Pa.

ZINC:

Associate referee: W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

FLUORINE:

Associate referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

LEAD:

Associate referee: P. A. Clifford, Food and Drug Adm., Washington, D. C.

MERCURY:

Associate referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

SELENIUM:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food and Drug Adm., New York City.

FRUITS AND FRUIT PRODUCTS:

General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

SOLUBLE SOLIDS AND EFFECT OF ACIDS ON SUGAR ON DRYING:

Associate referee: C. H. Badger, Food and Drug Adm., Washington, D. C.

ELECTROLYTIC TITRATION ACIDITY:

Associate referee: R. U. Bonnar, Food and Drug Adm., Washington, D. C.

FRUIT ACIDS:

Associate referee: B. G. Hartmann.

POLARISCOPIC METHODS FOR JAMS, JELLIES AND PRESERVES:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

CANNED FOODS:

General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

TOMATO PRODUCTS:

Associate referee: F. L. Hart, Food and Drug Adm., Buffalo, N. Y.

VINEGARS:

General referee: A. M. Henry, Food and Drug Adm., Atlanta, Ga.

ASH:

Associate referee: H. Shuman, Food and Drug Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

MEATS AND MEAT PRODUCTS:

General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:

General referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

GUMS IN FOODS:

General referee: L. J. Cross, Dept. of Dairy Ind., Agr. College, Ithaca, N. Y.

SPICES:

General referee: J. F. Clevenger, Food and Drug Adm., New York City.

MICROBIOLOGICAL METHODS—CANNED FOODS:

General referee: A. C. Hunter, Food and Drug Adm., Washington, D. C.

CANNED FISH PRODUCTS:

Associate referee: O. W. Lang, Hooper Foundation Medical Research, Univ. of California, San Francisco, Calif.

CANNED MEATS:

Associate referee: L. B. Jensen, Swift & Co., Chicago, Ill.

CANNED VEGETABLES:

Associate referee: E. J. Cameron, National Canners Assn., Washington, D. C.

CANNED TOMATO PRODUCTS:

Associate referee: B. A. Linden, Food and Drug Adm., Washington, D. C.

SUGAR:

Associate referee: E. J. Cameron.

FISH AND OTHER MARINE PRODUCTS:

General referee: H. D. Grigsby, Food and Drug Adm., Philadelphia, Pa.

SUGARS AND SUGAR PRODUCTS:

General referee: C. A. Browne, Bureau of Chemistry and Soils, Washington, D. C.

ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS:
Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

UNFERMENTABLE SUGARS IN MOLASSES:
Associate referee: W. L. Owen, Bayonne, N. J.

HONEY:
Associate referee: R. E. Lothrop, Bureau of Chemistry and Soils, Washington, D. C.

MAPLE PRODUCTS:
Associate referee: J. F. Snell, Macdonald College, Quebec, Canada.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:
Associate referee: C. F. Snyder, Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS (GENERAL):
Associate referee: C. A. Browne.

CHEMICAL METHODS FOR REDUCING SUGARS:
Associate referee: R. F. Jackson, Bureau of Standards, Washington, D. C.

REFRACTIVE INDICES OF SUGAR SOLUTIONS:
Associate referee: R. T. Balch, Bur. of Chemistry and Soils, Washington, D. C.

LEAD PRECIPITATE:
Associate referee: F. W. Zerban, N. Y. Sugar Trade Lab., New York City.

WATERS, BRINE AND SALT:
General referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

MINERAL SALTS AND EFFERVESCENT SALTS:
Associate referee: A. E. Mix.

CEREAL FOODS:
General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS:
Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

H-ION CONCENTRATION OF FLOUR:
Associate referee: George Garnatz, The Kroger Food Foundation, Cincinnati, Ohio.

STARCH IN FLOUR:
Associate referee: V. E. Munsey.

FLOUR-BLEACHING CHEMICALS:
Associate referee: Dorothy Scott, Food and Drug Adm., New York City.

CO_2 IN SELF-RISING FLOUR:
Associate referee: Howard Adler, Victor Chem. Works, Chicago Heights, Ill.

MILK SOLIDS IN MILK BREAD:

Associate referee: V. E. Munsey.

VISCOSITY OF FLOUR:

Associate referee: C. G. Harrel, Pillsbury Corporation, Minneapolis, Minn.

COLD WATER EXTRACT FLOUR:

Associate referee: H. C. Fellows, Bur. Agr. Economics, Washington, D. C.

ERGOT IN FLOUR:

Associate referee: J. H. Cannon, Food and Drug Adm., Chicago, Ill.

CATALASE AND PROTEOLYTIC ENZYMES:

Associate referee: W. S. Hale, Bureau of Chemistry and Soils, Washington, D. C.

COLOR IN FLOUR:

Associate referee: H. K. Parker, Novadel-Agene Corporation, Newark, N. J.

SOYA FLOUR IN FOODS:

Associate referee: J. W. Hayward, Archer-Daniels-Midland Co., Milwaukee, Wisc.

MACARONI:

Associate referee: W. F. Geddes, Board of Grain Commissioners, Winnipeg, Can.

WHOLE WHEAT FLOUR:

Associate referee: C. S. Ladd, N. Dak. Reg. Dept., Bismarck, N. Dak.

PHOSPHATED FLOUR:

Associate referee:

BAKING POWDERS AND BAKING CHEMICALS:

General referee: G. L. Bidwell, Food and Drug Adm., Washington, D. C.

FLUORINE:

Associate referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

LEAD:

Associate referee: P A. Clifford, Food and Drug Adm., Washington, D. C.

MICROCHEMICAL METHODS:

General referee: E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

ALCOHOLIC BEVERAGES:

General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

MALT BEVERAGES, EXTRACTS AND SIRUPS, AND BREWING MATERIALS:

Associate referee: J. A. LeClerc, Bureau of Chemistry and Soils, Washington, D. C.

DIASTATIC AND PROTEOLYTIC ACTIVITY OF MALT:

Associate referee: Stephen Laufer, Schwarz Laboratories, Inc., New York City.

CARBON DIOXIDE IN BEER:

Associate referee: P. P. Gray, Wallerstein Laboratories, New York City.

MALT EXTRACT IN MALT:

Associate referee: E. A. Siebel, 8 S. Dearborn St., Chicago, Ill.

MALT ADJUNCTS:

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Associate referee: L. V. Taylor, Amer. Can Co., Maywood, Ill.

ALCOHOL BY USE OF EBULLIOSCOPE:

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DETECTION OF ADULTERATION OF DISTILLED SPIRITS:

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WILEY MEMORIAL LECTURE. NO. VI

STRUCTURE AS AN APPROACH TO FOOD CHEMISTRY*

By ANDREW L. WINTON (Wilton, Conn.)

This lecture is an answer to an imaginary charge of chemical heresy. The accused pleads guilty, freely admitting that for the first quarter century of his active career he studied the sacred literature of that cult of botanists known as histology, albeit with the purpose of thereby aiding in the repression of fraud. He further admits that during the second quarter century, being thoroughly imbued with the spirit of liberalism, he and another, both relieved of official service, have departed still further from orthodoxy, advocating that every food chemist be christened at the Malpighian font before he is cloistered in research and further that high-school and college students, yes even children of tender age, be shown the visible miracles of foods, as well as drilled in the catechism of food composition. All this is admitted with the *sang froid* of a Russian revolutionist, scarcely hoping for mercy.

In extenuation it may be stated that the culprits have enjoyed no net financial gain from their extreme perfidy. On the contrary they have cringed under the pointed finger of chemical scorn and when all but excommunicated from the house of the lordly chemist have seen no finger beckoning from the tents of the wicked botanists.

Dr. Wiley was a heretic. He strayed from the straight and narrow path of chemical orthodoxy into the by-ways and hedges of medicine. In his éarly career he delved in the classics. All this heresy combined to better fit him for his supreme achievement. His knowledge of medicine gave him the proper grounding not only for combatting drug adulteration, but also for dealing with the hygienic aspects of antiseptics and other chemicals in foods. His classical training played an important part in developing his inimitably convincing power of presenting by both written and spoken word the unanswerable arguments against debasing the necessities of life. The precedent established by Dr. Wiley emboldens me to defend my own case, but before doing so let us consider what histology is and what manner of men worship at its shrine.

Malpighi and Grew in the seventeenth century are credited with being the fathers of histology, both plant and animal, but it was two hundred years later when, with the improvement of the microscope, vegetable histology was placed on a firm scientific basis. Undue emphasis was laid by the early histologists on trivial details and it was not until 1838 that Schleiden advanced the fundamental doctrine that the living cell is the unit of plant structure. Hartig, in 1837, definitely established that sieve tubes are the chief active elements of vascular bundles; soon after a num-

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ber of investigators took up the study of the form and arrangement of the elements of bundles, which constitute the remarkable plumbing systems of plants.

The third quarter of the nineteenth century was a period of intensive study of tissue forms and of substances, such as cellulose, lignin, and cutin, detectable by microanalysis in cell walls.

Turning from the cell walls to cell contents, it is quite possible that the ancients, equipped with crude lenses, observed that the large starch grains of cereals, legumes, and certain subterranean organs had definite form. If so, all records were lost during the dark ages. The Dutch botanist Leuwenhoek in 1716 recorded numerous observations on the microscopic characters of starch grains, but, as is so often true of discoveries, the facts attracted little attention until 1800 when Howard reviewed them, adding his own contributions. Soon after the discovery of the element iodine, in 1814, Gaultier de Chaubry found that this element dissolved in certain solvents gives a blue color with starch or starch paste, a reaction that ever since has been in continual use by histologists and chemists. Payen in 1838, and Nägeli, whose notable monographs appeared in 1858 and 1874, contributed greatly to the fundamental knowledge of starch as found in vegetable cells, and later a number of observers studied the characteristic forms of starches of different species.

Proteins, occurring as crystalloids in the so-called aleurone grains, received their share of attention, but the lack of reactions comparable in delicacy with the iodine test for starch and the presence of other substances in the grains have greatly hampered research in this field.

It may here be remarked that at no time in the past two or three generations has there been a dearth of papers on starch grains—Sjostrom's recent work is noteworthy—but relatively few investigators during that period had the courage to tackle aleurone grains, and as a consequence the statements in modern textbooks differ little from those published in the seventies and eighties. The aleurone grain is a complex, second only to the cell nucleus in its intricacies, that offers a fertile field for one trained in vegetable chemistry and histology, and equipped with modern instruments. If we knew more about the action of reagents, stains, and solvents on the crystalloids and other parts of these grains, we might better judge whether the apparently pure substances (such as Jones, following his illustrious predecessors Ritthausen and Osborne, is dissolving by protein solvents and separating by dialysis on a laboratory scale) are in fact unchanged constituents of the raw material.

From time immemorial man has expressed fatty oils from seeds and distilled essential oils from seeds, leaves, and bark. Early histologists went a step further, locating these constituents in the tissues and studying their formation, solubilities, and reactions. They adopted alkanna tincture as the standard test reagent for drops of fatty oil. They also observed

that solid fats, as existing in cells, often have crystalline form. Their pictures of the essential oil ducts, cavities, glands, and especially the exquisitely formed glandular hairs—fairy bottles as it were—occurring on the leaves of members of the mint family, have frequently been copied.

Such investigations paved the way for the fatty oil chemists and essential oil chemists, the former so well represented by H. S. Bailey and Jamieson, the latter by Chace, E. K. Nelson, and Albright, all now or formerly members of this Association.

Substances of the tannin group, long known to occur in tea, coffee, cocoa, and certain astringent fruits, have recently been shown by Caldwell, who distinguishes between tannin and non-tannin astringency, to be important factors in the flavor of apples. Progress in the study of tannin bodies has been aided by a remarkable set of color reactions. The purple color formed by certain tannins with iron salts was doubtless first observed by the woodman on his axe after he had cut into a bark rich in tannin.

The occurrence of calcium oxalate in a variety of crystalline forms, calcium carbonate as cystoliths, and silicious bodies as found in so-called stegmata has furnished subjects for numerous papers and for a classical monograph by Kohl that is rich in crystallographic data. Certain crystals of inorganic bases combined with organic acids, notably tartaric acid, are also visible, but most of the inorganic elements hide from view in combination, with no characteristic visual form.

Time will not permit of more than mention of numerous crystalline organic substances such as vanillin, coumarin, piperine, capsaicin, and hesperidin of natural foods, readily seen in microscopic preparations.

The early work in the study of cell wall constituents and cell contents, briefly outlined, has developed into what may be termed microscopic qualitative food analysis, although commonly known as food microscopy. It is more properly a branch of chemistry—visible chemistry—than of botany. Like cryptogamic botany—to this the chemist cannot lay claim—it has proved to be of real practical value, thus refuting the idea held by some that botany is merely a pretty subject for love-sick school girls.

A story is told of Torrey that illustrates the attitude of some early botanists. After an excursion in New York State, he engaged passage for himself and his bulky specimens on a Hudson River steamer. During the trip down stream he was busily engaged arranging his plants between drying sheets when a rustic fellow passenger accosted him with these words: "Mister, what good be them things?" Torrey continued his work without looking up but did deign to answer: "No good at all, no good at all; if they were any good I would not be bothering with them." Whether or not he lacked a sense of utility, it is fortunate for us that such patient workers spent their lives in laying a strictly scientific foundation on which later generations could build structures of real utility. They awakened an in-

terest in classification of plants in general and food plants in particular that no histologist or chemist, investigating vegetable products, can afford to ignore.

The last quarter of the past century witnessed an awakening of botanists to the practical importance of mycology. So great was the demand in this field that histology was neglected, although cytology claimed the attention of a picked few. In the meantime the pharmacognocist and the food microscopist had come into prominence owing to the passage of laws for the suppression of fraud. Berg's treatise on the histology of drugs, published in 1865, illustrated with beautiful lithographs, was followed by a score of other treatises in different languages. Vogl, Moeller, and Tschirch wrote in German, Macé and Collin in French, Greenish, Kraemer, and Youngken in English. Many vegetable drugs, also a few foods sold in powder form, were studied, and their microscopical features were given due consideration.

Little by little food microscopy split off as a distinct subject, deserving of special papers and treatises, but the detection of fraud was the dominating purpose, and foods that were not regarded as adulterable (such as many fresh fruits and vegetables), or suitable for adulterants, were commonly ignored. The variety of food products examined was accordingly limited. Spices, tea, coffee, cocoa, the common cereals, and certain oil seeds were the most important. When, as a result of the stringent enforcement of laws and regulations, these, as well as the somewhat greater variety of food products that were examined by the food chemist, ceased to be subjects for the alarmist, there was no need of a force, growing in size with the population, to protect the consumer from fraud. The field for both types of workers would have been indeed limited were it not for the remarkable fact that when the era of food frauds was on the wane we were entering or rather reentering into an era of food research in which both chemists and histologists have unlimited opportunities.

On this occasion I shall confine my words to the nature of the work proper to the research histologist who does not stress any particular practical application, but is desirous of cooperating with the research chemist in extending our knowledge of food constituents.

Two people, working with limited apparatus on 300 to 400 natural products in a corner of a barn, can do little more than map out the terrain in which future work by others may be expected. Such a mere scratching of the surface, however, brings to light innumerable points of interest which one is often tempted to carry to a logical limit, but to do so would imperil the prearranged plan to cover the whole field.

In what follows, some of the statements have been abundantly demonstrated, others are speculative. Some are leads that may or may not be worthy of consideration; at least they call attention to a few gaps in the literature that should be and can be filled. In some cases the microscope

does not reveal constituents found by the organic chemist, in other cases our knowledge, derived from a study of the histology, is far in advance, even in the matter of chemical constituents, over that available in the purely chemical literature. Above all, the data illustrate that the histologist can often obtain an insight into the chemical nature of natural products, and even gain an idea of percentage composition, that compares favorably in practical value—yes, in chemical value—with that derived from the study of widely varying results of published analyses. Just as each atom is now regarded as a little solar system, so each seed, fruit, root, tuber, or leaf is an enchanting universe about which, even with our present limited methods, more can be learned, and with improved instruments and technique an almost limitless extension of our knowledge may be expected.

Cereals, on which civilized man chiefly lives, deserve our first attention. In the United States corn is king, but he is an indirect ruler through the hog; wheat is really the great monarch and he deals directly with his subjects. Much has been written on the chemistry of cereals, but there is so much still to learn. Analyses or investigations on the whole kernels give a confused idea, those on the parts bring us nearer to the fundamentals. We can separate by hand—laboriously to be sure—the true bran, the germ, and the endosperm. The term bran is so indefinite as to render much published data of little value. Some milling processes run together germ, true bran (I use this term arbitrarily for fruit-coat, seed-coat, and hyaline layer), aleurone cells, and more or less starchy endosperm; others separate bran coats from germ, eliminating so far as possible all endosperm tissues. Patent flour and certain granular breakfast cereals are nearly pure starchy endosperm. All milling processes squeeze more or less fat (oil) out of the germ, so it is uncertain how much oil actually belongs to the starchy endosperm. The amount there certainly is small, perhaps not more than a trace.

As seen under the microscope, no starch is present in the aleurone layer; this constituent appears in the layers beneath, the size and number of the grains increasing inward. Cobb showed how by cautious heating of sections in water the protein (gluten) matrix, in which the starch is embedded, may be coagulated and rendered evident.

The cell nuclei complete the visual picture of the inner endosperm tissues, in which starch is the predominating constituent. The picture of the germ is radically different; each cell is rich in oil, but it also contains invisible protein and a nucleus in which nucleic acid abounds; starch is entirely absent. As shown by staining, the cell walls of some of the bran coats are composed of true cellulose, others of lignin. In the cleft are interesting tissues long ignored, but subjects for extended research. There are present vascular bundles with spiral vessels, sieve tubes, and other elements, a colorless cartilage-like strand consisting of perisperm or body

of the ovule left over after development, and a thickened dark-colored strand of seed-coat tissues.

Each part of the kernel deserves years of research, histological and chemical. Not the least part of the labor will be the dissection and separation of the parts. A true investigator will not say he has no time for this. I remember an illustration in the *Fliegende Blätter* of nearly fifty years since. A portly sweating gentleman is shown expostulating with the music master, who has gone into a passion over the insistence of the gentleman's daughter on making a full rest instead of a half rest. "Ach," exlaimed the indulgent parent, "Meine Tochter hat die Zeit." Only an aristocrat in science, with time to secure the best possible separation of the morphological parts, should attempt a chemical investigation involving the nature of the constituents. Grinding the whole kernel is as unscientific as to make no attempt before analysis to separate several minerals in the same matrix.

Much can be learned from a study of the cereals in different stages of ripeness. The starch grains of Indian corn in the milk are round, and they float in the cell liquor. In the floury part of the mature endosperm they are still round, but in the horny part, owing to crowding, they are polygonal. Pigs' bladders would be polygonal if they were crowded together.

Do not think because a cereal kernel is small and millions of dollars have been spent on its study that there is no more to do. The work has hardly begun, yet little can be expected without a new approach. Within the past few months in a study of certain breakfast cereals we unexpectedly ran across structural peculiarities that materially enlarge our knowledge of the original kernels. With our present technique we see only the most abundant constituents of the cereals, but I am confident that by color reactions and other microchemical tests we shall eventually be able to locate even the vitamins.

Buckwheat and wild buckwheat (black bindweed), although far removed in the scheme of classification from cereals, have certain points in common with them. They have a bulky, starchy endosperm and an oily non-starchy embryo. They have received scant attention. Our meager knowledge of the proteins of buckwheat is due in part to difficulties of separation.

Seeds of the closely related pink, goosefoot, and amaranth families, notably cockle, lamb's quarters, and pigweed, all, like wild buckwheat, weed seeds produced in enormous quantities in grain fields, furnish a particularly alluring but almost untrodden field for the study of starch and proteins. In these the starch grains, present not in endosperm but in perisperm, are exceedingly minute. So far as noted, the starch and proteins of black pepper, present in the bulky perisperm, have not been studied chemically. The wider variety of seeds studied, the greater the likelihood of a more fundamental knowledge.

Turning to economic leguminous seeds of which there are many, grouped in twelve subfamilies, we find that some are rich in starch—almost as rich as the cereals—and others are starch-free but rich in proteins. The starch grains, although varying somewhat with the subfamily, are more or less bean- or kidney-shaped, with what has long been known as an elongated hilum. With polarized light, this so-called hilum connects the points of two V's. Since only chemical microscopists are usually versed in crystallography, we perhaps may be excused for long violating the rules by assuming an impossible structure wherein the lines of the polarization crosses do *not* cross. As a matter of fact they *do* cross, but at highly excentric hilums of two or three individual grains joined in a row, often with a mechanical rift between, giving the appearance of a single grain with an elongated hilum. All transitions are evident in immature seeds.

An explanation of differences between smooth and wrinkled peas was revealed by the microscope. There is a comic song that runs: "No matter how young a prune may be, it's always full of wrinkles." We may say that no matter how young a wrinkled pea may be, it's always full of wrinkles, whereas no matter how old a smooth pea may be, it's always free from wrinkles. The starch in fully ripe wrinkled peas is like that in unripe smooth peas. In other words, the wrinkled pea may be fully ripe, but the starch has not reached full development.

A parallel case is that of sweet corn which, unlike field corn, has a wrinkled surface when fully ripe. Here again at full ripeness of the seed the starch is not fully developed but resembles that in immature field corn. Hanausek in one species of the corn group and Weatherwax in another observed a remarkable degree of immaturity as evidenced by the color with iodine solution which, instead of being blue, was coffee-colored. Because the starch grains do not reach full size and consequently the percentage of starch is low, it follows that the protein is proportionately high as shown by numerous analyses in the literature. The carbohydrate chemist may well turn his attention to sweet corn, supplementing the valuable results of Straughn and Church on total sugars secured some forty years since; the protein chemist may well work on peas of the two types and on sweet corn.

Progress in the chemistry of cruciferous seeds, notably the rapes and mustards, has not in recent years kept pace with that in the histology. The microscope reveals in some species remarkable mucilaginous deposits, which with water burst out of the cells, and in charlock a coloring matter, reacting with acid chloral hydrate, that awaits investigation by the pigment chemist. Guinard located the seat of the myrosin, that peculiar enzyme that acts on sinigrin forming volatile mustard oil, in special cells. We see proteins in the cells, but we know little of their chemistry.

The gossypol of cottonseed has been treated during recent years in numerous articles. The location of this principle is in the resin sacs, known to histologists since Hanausek's first paper.

An illustration of the lack of cooperation between chemist and histologist is the surprising failure of my friend David Wesson, the world's authority on cottonseed oil, to have noted the structure of the seed until shortly before his death. The matter came up at our house, where he was always a most welcome guest, and he hastened to incorporate the facts in a paper he was preparing.

Bigelow, Gore, and Howard employed chemical and microscopical technique over thirty years since in their study of the development of apples, the results of which have lost nothing in value with time. The increase in carbohydrates during ripening was not only found to be accompanied by a decrease in acidity, but the starch visible during the early stages disappeared with a corresponding increase in sugars during the later stages. Since then results have been obtained with other fruits by workers in the old Bureau of Chemistry and elsewhere. The work of Browne in Pennsylvania and of Caldwell, Culpepper, and others in the Bureau of Plant Industry deserves special mention.

Pectin is today a magic word for fruit chemists. Braconnot isolated a pectin over a hundred years ago, and early histologists learned that cell walls, whether with cellulose or lignin layers or both, always have a middle lamella separating them, but chemist and histologist did not get together until Mangin in 1888 located the seat of pectin in the middle lamella. It was well known that cells of succulent fruits and vegetables hold together until full ripeness when they separate as sacs, but no one had previously followed the simple logic of regarding pectin as the cementing agent.

Pectin has been shown to constitute the outer lamella of each cell wall, even when not in contact with another cell, hence on surfaces adjoining intercellular spaces it exists in potential form like the glue on a postage stamp. This point has an application in the study of the interlacing strap-shaped cells of orange albedo to which Berg called attention in 1865.

Berg overlooked, but Tschirch observed, the chemical substances visible in other parts of the orange. The essential oil of the rind shows up as minute drops in special cavities, causing the small elevations on the surface. In other parts of the rind, needle-shaped hesperidin crystals and prismatic calcium oxalate crystals are evident. The vesicles of the pulp—the part we eat—are so to speak compound juicy hairs containing rosette crystals of calcium oxalate and color bodies held in the acid saccharine liquid. The seeds go the Dionne quints three better for in a single seed as many as eight embryos may be counted, each containing clearly visible aleurone grains and fatty oil, all enclosed within a seed-coat of remarkable structure.

The strikingly different structure of the seed-coats of common citrous fruits, long unnoticed, is believed to have a bearing on botanical classification. A great food problem is to free the rich store of nutrients in citrous seeds from bitter principles. Other similar problems are to make the huge starchy seeds of the avocado and the mango palatable.

In the matter of sugars in fruits so well handled by Browne, of acids worked on by European chemists, by Bigelow, Dunbar, and Bacon, and more recently by E. K. Nelson in this country, and of odorous constituents on which Power, Chesnut, and Nelson have done so much, the histologist must do obeisance to the organic chemist. No practicable crystallographic or micro-tests are available.

As regards calcium oxalate in the remarkable crystalline forms occurring in foods, particularly in fruits and vegetables, there is still much for the histologist and the chemist to do. I believe that we should have more complete evidence that the crystals generally assumed to be calcium oxalate really are that. Crystal clusters with sharp angles must be chemically or physically different from those with blunt angles, even if both give calcium and oxalic acid reactions. In solving these problems, we need the aid of the crystallographer and the microchemist. Chamot has laid a firm foundation.

Needle-shaped crystals, known as raphides, are of interest because they are accused of causing sore mouth on eating raw pineapple. To them is also attributed the acridity of certain varieties of taro, dasheen, and true yams, so painstakingly studied by R. A. Young. These raphides perhaps may be eliminated by breeding.

Of the various groups of vegetables grown throughout the world in which the microscope opens up a wealth of detail long neglected, the roots of four families, the beet, the turnip, the carrot, and the composite, are especially worthy of notice. The usual proximate analyses, whether on the fresh or dry basis, bring out little or no distinction of one root from the others, but a study of the structure, coupled with the results of polariscopic analysis, is most illuminating.

The explanation of the rings in the beet was found by Wiesner seventy years since. Each ring has a true cambium layer with active cells dividing tangentially, thus differing from the concentric rings of trees, within the single cambium layer, which consist of dead xylem cells only. The sucrose is located chiefly in large parenchyma cells between the rings. Masses of so-called crystal sand, chemically calcium oxalate, occur in special cells in the phloem.

The turnip family, like the beet group, is characterized by an abundance of sucrose. Allyl mustard oil, present only in small amounts in turnip and rutabaga, but in larger amounts in horse-radish, is the flavoring substance. Yellow turnips and rutabagas contain color bodies. Starch is present in both during the growing season and in horse-radish at all times, even after wintering in the ground.

Still more noteworthy are roots of the parsley family. These contain starch during the growing season, but it disappears at the end of the season or after wintering in the ground. Essential oil occurs in minute drops in special cells surrounded by cells with aleurone grains. A peculiar

type of elongated cells with cartilage-like walls, known as substitute fibers, is characteristic. Carotene crystals occur in orange or deep yellow varieties.

Composite roots are the most strikingly differentiated of all common roots. In these there is no starch but an abundance of inulin, which readily crystallizes in alcoholic material. Ramifying through the tissues are conspicuous branching latex tubes filled with latex grains in a watery liquid, hence the milky juice.

Other groups of vegetables and fruits are characterized by the occurrence of inulin and (or) latex. The Jerusalem artichoke is a root-tuber rich in inulin, from which McGlumphy, Dykins, and others prepare by hydrolysis commercial levulose. Latex is seen under the microscope not only in the roots and tubers but also in the leaves of composite plants, notably lettuce and endive, as well as in various tropical fruits such as the sapodilla. The tree that bears the sapodilla also yields, by tapping the bark, latex, which is the basis of chewing gum.

The common potato is a marvel of structure. Ask a domestic science student to remember that the tuber contains upward of 20 per cent of starch and about 2 per cent of protein and the chances are she will forget it by examination time, but if you show her the cambium ring on a cut surface and then under the microscope the protective coats of orderly arranged cork cells, the cubical protein crystals, the starch grains resembling glistening shells on the sea shore, and the exquisite lace-like xylem layer with spiral and reticulated vessels of fanciful design, information is imparted that will change paring potatoes from drudgery into a scientific pastime.

If the organic chemist enjoys a monopoly of soluble colors such as the anthocyanins, whose constitution yielded to Willstätter's persistence, he must share with the histologist the carotinoids that appear in definite crystalline form in vegetable cells. We, who saw carotene and lycopene over fifty years since, are enthusiastic over Karrer's achievement in placing the group on a firm chemical basis. A ready means of showing that carotene is closely related to vitamin A was allowed to pass unnoticed until demonstration was achieved in other ways. I refer to the presence of carotene crystals in the ordinary orange-colored carrot and their absence in white carrots. Had the vitaminist been versed in vegetable histology and the lore of seedmen's catalogs, he could hardly have failed to embrace that opportunity.

In the matter of vitamins in general, neither vitaminist nor histologist, but the organic chemist deserves the crowning glory. To him belongs the praise for leading us out of the empirical Egypt of hieroglyphics into the promised land of chemical harmony and synthetic felicity. All honor, however, is due the physicians who, long before vitamins were thought of, extolled the virtues of coarse bread, fruit, vegetables, and clean milk, and

the vitaminists who, beginning with Falk, paved the way for the organic chemists. Let us hope the microscopists—members of the American school of Chamot—may yet take an active part in this great nutritional movement by developing methods for detecting and locating the different vitamins in foods.

Passing over the amylodextrin of mace and the erythrodextrin of the durian aril, let us consider for a moment the carbohydrate deposited as hard but digestible reserve material in the cell walls of vegetable ivory, coffee, date stones, and persimmon seeds. Seeds of this type are a group quite as strongly characterized as starch seeds and oil seeds, but no appropriate designation has yet been suggested. The term "hemicellulose" seeds may meet the need until we know more of the individual substances of cell walls. It is of interest that several seeds of this type with thickened cell walls yield on roasting coffee-like products, which in the form of an infusion resemble more closely real coffee than do those made from cereals or oil seeds. In addition to vegetable ivory and date stones, which have long been so used, persimmon seeds, properly roasted, make in our experience a quite acceptable beverage. Whether it is practicable to develop varieties adapted for this purpose or whether the coffee fiends will accept any well flavored beverage without a "kick" is another matter.

At this time, when at last investigators are practically agreed as to the structure of the chlorophyl molecule and Hans Fischer is sanguine of achieving its synthesis, all vegetables rich in chlorophyl, especially the so-called leaf vegetables, are uppermost in our minds. It behooves histologists today to pick up the thread dropped by earlier workers with the hope that there may be found visible differences between the forms of chlorophyl *a* and chlorophyl *b*, not overlooking the nature of the residual grain after extraction of the pigments. Here again improved apparatus and special technique suggested by discoveries in the chemical laboratory may lead to important developments.

Perhaps I am a poor exploiter not to embrace this opportunity to bring my subject to a climax—to portray on a huge canvas our histological goddess enthroned, her faithful microscope at her side looking up devotedly into her eye, surrounded by her vassals, each appropriately clothed in Raphaelian colors—xanthoproteic yellow, iodine-starch blue, alkanna fat red, chlorophyl green, carotene orange, and iron tannate purple, but that is the opposite of the picture I would paint. I would show histology as a handmaiden officiating at the birth of an idea and later helpful, but stepping aside while the organic chemist isolates, identifies, and finally synthesizes the actual constituents.

It is folly to exaggerate the achievements of different groups. Each has a task to perform. Great ability may be necessary for solving a problem of trivial importance, whereas mediocrity may suffice for an all important achievement. All should work together, the chemist listening to the his-

tologist and the histologist learning more of chemistry. Above all there should be a greater coordination of the applied groups of food science. Let us combine to make bromotology—a seldom used, queer sounding although expressive term—a grand comprehensive subject with a broad scientific basis.

The different workers that have studied foods in recent years have more often started with a desired application—whether nutritional, forensic, industrial, or agricultural—and too often have forgotten that the really practical is what we call pure science. Food science has not developed soundly from the trunk, but as it were from the tip of the branches. Can you imagine, for example, qualitative and quantitative chemistry developing in this manner, one group working on methods for the detection of adulteration of precious metals, some seeking solution of mining problems, and others of manufacturing problems, all indifferent to fundamental knowledge discovered by the pure scientist? Specialties we must have, but let these branch off in orderly harmonious manner from the grand mother science.

As a consequence of the haphazard development, we have important scientific papers scattered through widely divergent literature—König's monumental work at the start stressed animal feeding, later recognized human nutrition, and still later inspection chemistry. Since funds have been more often forthcoming for inspection work than for other branches, the literature on the detection of frauds and the diagnosis of mixtures has predominated. To some extent the inspection chemist has used the nutrition chemist's methods for protein, fat, and fiber, but the nutrition chemist has not so often been interested in the methods of the inspection chemist. The industrial chemist sometimes has followed the lead of both and sometimes has advantageously struck out for himself. All groups today are coming to appreciate that the organic chemist must be depended on to lay the foundation on a rock. He is a true fundamentalist. Ritthausen, Tollens, Emil and Hans Fischer, Willstätter, and Karrer are among the high priests. To them and others of their kind we must turn for real inspiration. In this short list I mention only pioneers of the German school. American chemists are now coming to the front. At this time one American deserves mention, first, because his work of thirty years under difficulties on one of the most fundamental problems of the generation reached fruition during the past year, the isolation and synthesis of vitamin B, and second, because he was once one of us: R. R. Williams.

May such American names as Williams, Conant, Treat Johnson, Hudson, Levene, Jones, Nelson, and Van Slyke the younger, be an inspiration to our younger investigators.

To those research workers with a botanical and histological background I can only say: "Look well to your laurels for the mantle of Harz, that master of seed science—spermatology if you prefer the word, has now

fallen on a Japanese investigator, Mantaro Kondo, whose two volumes, the only modern authoritative work on the subject in any language, are in Japanese, which none of us can read."

I have mentioned some of the outstanding names in Europe and America. I wish also to mention with no little emotion three of the outstanding men who have worked with me and are attending this meeting: E. M. Bailey of Connecticut, A. E. Paul of Chicago, and H. S. Bailey, formerly of Washington.

PRESIDENT'S ADDRESS*

By HERMAN H. HANSON (State Board of Agriculture, Dover, Del.)

It was just thirty years ago that I attended my first meeting of the Association of Official Agricultural Chemists, having become a member four years previous to that time by virtue of appointment as assistant chemist in the Maine Experiment Station. That meeting was held in George Washington University, in this city. The meeting of the following year, at the Jamestown Exposition, near Norfolk, was also attended. The sessions were held in the "Inside Inn." At these and early subsequent meetings, it was my privilege to see in action many of those earlier members whose careful and painstaking work contributed so materially to the world-wide recognition of the Official Methods. There were Hopkins, who, in his presidential address in 1906, took violent exception to certain theories of soil chemistry then being expounded; Frear, to whose remarks, couched in the most precise and expressive terms, it was always a delight to listen; Davidson, who seemed always to be rushing from one committee to another with reports in preparation; Ross, another seldom seen without a great sheaf of papers; Van Slyke, whose wide knowledge of subjects agricultural always insured attention; Ladd of paint fame and afterwards United States Senator; Snyder, the cereal expert; Armsby, who built the respiration calorimeter; and there was Dr. Wiley, to whom all turned for inspiration and leadership. There were others, of course, and a few of them are still regular attendants at these meetings: Carpenter, Huston, Gascoyne, Hand, Bigelow, Patterson, Fraps—I cannot name them all here, but these and others of like caliber were the men from whom I received my first impressions of this Association. I listened to their reports and discussions with the admiration and deference of a neophyte, grateful for the privilege of being a most unimportant member of one of the most important scientific organizations in the country, formulating the methods of chemical analysis accepted as official in the highest courts and numbering in its membership many of the leading chemists of the country. I confess that I have never quite grown away from those first impressions, nor, indeed, do I wish to outgrow them. My association with the members of this organization has been a valuable and happy experience and the many friendships formed are deeply cherished; and I come before you today with humility, fully conscious that I cannot bring any learned discussion on researches conducted or discoveries made, but only a few casual observations.

Compared with the meetings of recent years, those of thirty years ago were attended by only a small group, the subjects for discussion were few, and there were no sectional gatherings. The Book of Methods used by the speaker was Bulletin 46 (Revised), a paper-covered pamphlet of

* Presented Monday afternoon, November 30, 1936.

eighty-six pages. The difference between that pamphlet and the volume of more than seven hundred pages just issued represents the growth that has taken place during that period. This growth of our Association, this expansion in our activities, is the natural result of the rapid changes in the requirements of civilization brought about by scientific discovery and achievement.

In those earlier days every member of the Association was interested in all its activities, and if a new method, or a modification, was proposed, the proposer had to be prepared to defend it against all comers. There were real discussions then, and if there were flaws either in a new proposal or in an argument, they were rather sure to be pointed out. Such general and all-inclusive discussions are impossible now. There are too many subjects and we resort to group meetings. And in the groups there are specialists on whose particular studies and researches we must of necessity rely. How many of the 425 registered at the meeting last year have made or expect to make a determination of lignin? How many have the facilities for conducting the biological tests to show the vitamin D potency of carriers? How many will ever analyze for "less common metals in plants" or the "diastatic value of flour"? How many are interested in malt beverages? (Perhaps I should have left that one out.) Yet these are all methods of prime importance in some phase of the broad field of agriculture and in the activities of the control chemist, and each of the many subjects on our program is properly listed there. Even the fertilizer chemist is no longer satisfied with his time-honored nitrogen, phosphoric acid, and potash, but he must discuss magnesium, manganese, and residual acidity—and the end is not yet.

It is true that there are discussions within the groups, but there are nearly as many subjects now in some of the sections as there were a few years ago before the whole Association. At the first meeting following the World War, only seventeen years ago, there were fifty-seven subjects of reports and papers. On our program for last year we had one hundred and forty-eight. At that meeting in 1919, in addition to the general sessions, there was but one sectional group, that for drugs, which had six subjects for consideration. Last year there were five sections, and this year a like number, in addition to the general one, and the drug section had before it twenty-two topics. With all the new subjects for investigation added to our program, seldom is one definitely finished or discontinued. Only last year we adopted as official an important modification of the method for determining that old original—potash.

Our growth is gratifying but it has its embarrassments. Thus far we have not increased, at least for many years, the time devoted to the meetings but have met the situation by dividing into sections. This plan has its disadvantages as it often happens that members would like to be in more than one place at the same time. We have planned for five new

subjects for the *Book of Methods*, each to occupy a chapter. They appear in the latest edition by title only, but at the earliest possible moment real chapters should take their places. We should consider very carefully before adopting as our official methods those sponsored by any other group or organization without first subjecting them to the test of our usual collaborative procedure. This will mean the appointment of several new referees and much cooperative work on the new lines. This would entail many new items for report on our program and either lengthen our meetings or necessitate new sections. It seems obvious that more sections are not desirable if it is possible to avoid them.

A moment ago I spoke of specialists on whose studies and researches we must rely. With the increase in new products, the utilization of waste material, the use of new chemicals and reagents accompanying the rapid expansion in the chemical field, specialization is necessary; but new methods of analysis developed by specialists should be given sufficient trial by collaborative work to insure that they are practical under the usual laboratory conditions before they receive the stamp of approval from our Association. In this connection there is another feature which should receive attention. Before any new method is accepted in its final form, it should be so plainly and unmistakably worded that it may be used with confidence by others who, although they may be analytical chemists with thorough general training and considerable experience, may not have had that particular experience of the specialist who formulated the procedure. Occasionally a method is involved and complicated. To the one who invented it, it is quite clear but for the benefit of the beginner each step should follow the preceding in logical sequence, quantities named should be exact, chemicals and reagents used should be unmistakably specified. In other words, every turn of the trail should be plainly marked.

Judging from the past and viewing the prospect ahead, we should at once proceed to the preliminary work on the five missing chapters of our compilation of official methods, while at the same time we continue that which is already in progress. This will mean new referees and more collaborators. It will mean more reports and more discussion, and it will probably mean an extension in the near future of the time for our meetings.

The future, also, will undoubtedly bring to us subjects not at present contemplated. If they come within the true province of our organization they will be adopted as a part of our program, but it will be unwise to attempt to cover too much ground. At present the services of this Association are not duplicated by any other and the recognition it receives is gratifying, but if we spread our activities too widely the coverage will be thin.

The objects as set forth in the Constitution of the Association of Official Agricultural Chemists of North America are:

1. To secure, devise, test and adopt uniform and accurate methods for the

analysis of fertilizers, soils, foods, feeding stuffs, dairy products, insecticides and fungicides, and other materials relating to agricultural pursuits; also medicinal products; caustic poisons; paints, paint materials, and varnishes.

2. To secure uniformity in the statement of analytical results.

3. To conduct, promote, and encourage research in chemistry in its relation to agriculture.

4. To afford opportunity for the discussion of matters of interest to agricultural chemists.

Very much the largest proportion of the time of our meetings is devoted to the first of these four objects. It is for the methods of analysis that the Association is universally recognized, and it is for the purpose of presenting, discussing and learning about methods of analysis that most of the members attend these sessions. However, there are several committees of the Association working under the second and third objects, "to insure uniformity in the statement of analytical results," and "to conduct, promote and encourage research in chemistry in its relation to agriculture," whose reports are heard by comparatively few of our members, and do not receive the credit to which they are entitled, as the reports are always received during the closing moments of our meetings.

The American Public Health Association devotes a considerable portion of its activities to the subject of milk and covers all phases of the production and distribution of this important food. A committee of this Association has the duty of conferring with the American Public Health Association on standard methods of milk analysis, and that Association has adopted in toto our methods. Our committee is in constant touch with that organization, keeps us informed of its needs, and transmits to it our latest developments, thus contributing to efficiency and uniformity.

The "Report of the Committee to Cooperate with Other Committees on Food Definitions" is always short, and always a report of progress. It gives little indication of the time, including innumerable hearings and discussions, devoted to the task of formulating the definitions which, when satisfactorily completed, are promulgated by the United States Secretary of Agriculture. Our representative on this committee has always faithfully and conscientiously attended to the duties imposed, and his short report to our Association gives scant hint of its importance.

In 1920, the Crop Protection Institute was formed under the auspices of the National Research Council. Its purpose "was to find better means of utilizing the extensive equipment and facilities of State Agricultural Experiment Stations, the universities and other agencies, and the expert knowledge of the staffs of these several institutions in cooperation with the manufacturers of insecticides and fungicides, and those who make the equipment necessary for their utilization." We have representatives of the A. O. A. C. on the Board of Governors of this Institute, whose reports we annually receive and file, more or less as a matter of routine. "The progress of the institute," I quote from a late report, "has been gradual

but nevertheless steady, and the reports issued from time to time show great accomplishments with commendable economy. New copper fungicides, oil sprays, the possibility of pyrethrum culture in the United States, preliminary studies of organic compounds of promise as insecticides and fungicides, development of a better spray for codling moth and other insects, new fumigants, new contact insecticides, extensive sulfur investigations, development of insecticides and fungicides utilizing sulfur and carbon bisulfide, research on plant extracts that might be useful, improved methods of applying a variety of sprays—these indicate the types of problems which engage their attention."

Our representatives deserve great credit for their work in the Institute, the purpose of which covers one phase of our third object, "to conduct, promote and encourage research in chemistry in its relation to agriculture."

In conclusion, may I express my appreciation for the honor of presiding at this fifty-second annual meeting. We cannot with any certainty predict the future; we can only judge by the past. Our accomplishments have been extensive and important, and we may confidently expect that a similar progress will continue. If we are to complete satisfactorily the new lines of work already projected, and be in a position to meet the new developments that will arise, many new investigators must be enlisted. With the increasing interest in our problems, as evidenced by our increased attendance for the last few years, we may have no doubt that the workers will be forthcoming as needed. May I extend an official hearty welcome to all members and friends present, and express the hope that these sessions will be most pleasant and profitable.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY

WEDNESDAY—AFTEROON SESSION

REPORT OF EDITORIAL BOARD

I shall depend upon the chairmen of the several editorial committees to make detailed reports of the work of the Editorial Board. I should like to say that we still have under consideration the advisability of printing certain material in the form of monographs to be distributed to the members. As you will see later on from the reading of the treasurer's report, the financial status of the Association justifies further expenditures for publications. However, it has not yet been possible to work out a plan for handling this material. We will now have the detailed reports of the committees.

W. W. SKINNER

REPORT OF EDITORIAL COMMITTEE OF THE JOURNAL

The satisfactory financial status of our *Journal* reported by our secretary-treasurer is accompanied by gratifying additions to the subscription list, continued increase in the number of pages published, and a bigger sale of advertising space. Book reviews have been published in greater number. By all signs our *Journal* is growing up. Outstanding contributions to the literature on analytical chemistry appearing in its pages have attracted world-wide attention. We have definitely passed the established estimate of 600 pages per volume. Contributed papers this year accounted in good measure for the increase in size. The advance should be maintained, and the interest of potential contributors should be solicited. The finances permit some further expansion, which the Executive Committee has authorized. In reviewing the numerous chemical publications one often finds articles dealing with subjects peculiarly suited to our *Journal* submitted by authors who are eligible for membership in our Association. It is our expectation to bring to the attention of the many workers in agricultural chemistry the opportunity our *Journal* offers for the publication of contributions in fields of interest to our readers. It may be possible also to encourage the writing of special articles on critical studies of methods of analysis of specific products or constituents. As gratifying as the progress is, it is not without some lack of accomplishment to check our pride. Our *Journal* has not appeared with the promptness your Committee promised at the last meeting. Now that the task of publishing

Methods of Analysis is successfully completed, the efforts of the editorial office can be more fully devoted to the *Journal*, and we have every reason to believe prompt publication will be a reality this year.

H. A. LEPPER

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF METHODS OF ANALYSIS

The 4th edition of *Official and Tentative Methods of Analysis* was carried out in accord with the outline presented in the report of your Committee at the meeting last year, *This Journal*, **19**, 40 (1936). The volume is now in your hands. Its preface describes in adequate detail the additions to subject matter and other changes that have been made, and no further elaboration is necessary here.

As the scope and volume of our work increases, some expansion in the volume of the text is inevitable. The worth and usefulness of our book is measured by the scope and reliability of its subject matter. On the other hand, we must guard against undue bulk in order that the cost of the volume to those who need it may be kept at a reasonable sum. This double objective is a challenge to the ingenuity of each succeeding revision committee. The excellent 3rd edition introduced a number of space-saving devices. These have been retained and somewhat extended in the present edition, yet the new volume contains nearly one-fifth more pages than the edition immediately preceding.

The editorial plan and practice of our Association emphasizes accuracy, clarity, and brevity as cardinal virtues in the description of methods. By and large our methods reflect that policy in an admirable degree, but occasional lapses may still be found. Observance of the essentials just named properly begins with referees and associates in recommending methods for adoption. A happy combination of these descriptive features requires no little skill and thought but such effort is not lost; it adds tremendously to the value and usefulness of our text.

The generous cooperation of the Association's referees, associate referees, and collaborators has been appropriately acknowledged in the preface to the new edition, and in that testimonial the Revision Committee heartily concurs. Without help from these sources our task could not have been accomplished. We hope that the text will serve your increased needs more fully than has been possible heretofore, and that it will measure up to your expectations.

For the Revision Committee,

E. M. BAILEY, *Chairman* MARIAN E. LAPP
J. W. SALE G. G. FRARY
L. E. WARREN H. A. LEPPER

Approved.

No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

Final Adoption as Official

1. AIR-SLAKED LIME

Air-slaked lime is a product composed of variant proportions of the oxide, hydroxide and carbonate of calcium, or of calcium and magnesium, and derived from exposure of quick lime.

2. PULVERIZED LIMESTONE (FINE-GROUND LIMESTONE)

Pulverized limestone (fine-ground limestone) is the product obtained by grinding either calcareous or dolomitic limestone so that all the material will pass a 20-mesh sieve and at least seventy-five per cent (75%) will pass a 100-mesh sieve.

3. GROUND LIMESTONE (COARSE-GROUND LIMESTONE)

Ground limestone (coarse-ground limestone) is the product obtained by grinding either calcareous or dolomitic limestone so that all the material will pass a 10-mesh sieve, and at least fifty per cent (50%) will pass a 100-mesh sieve.

4. GROUND SHELLS

Ground shells is the product obtained by grinding the shells of mollusks so that not less than fifty per cent (50%) shall pass a 100-mesh sieve. The product shall also carry the name of the mollusk from which said product is made.

5. GROUND SHELL MARL

Ground shell marl is the product obtained by grinding natural deposits of shell marl so that at least seventy-five per cent (75%) shall pass a 100-mesh sieve.

6. ACID-FORMING FERTILIZER

An *acid-forming fertilizer* is one that is capable of increasing the residual acidity of soil.

7. NON-ACID FORMING FERTILIZER

A *non-acid forming fertilizer* is one that is not capable of increasing the residual acidity of the soil.

Second Reading as Tentative

1. DOLOMITE

Dolomite is a mineral composed chiefly of carbonates of calcium and magnesium in substantially molar proportions.

2. PRIMARY FERTILIZER COMPONENTS

Primary fertilizer components are those at present generally recognized by law as necessary to be guaranteed in fertilizers, namely: Nitrogen, phosphoric acid, and potash.

3. SECONDARY FERTILIZER COMPONENTS

Secondary fertilizer components are those other than the "primary fertilizer components" that are essential to the proper growth of plants and that may be needed by some soils. Some of these components are calcium, magnesium, sulfur, manganese, copper, zinc, and boron.

4. BAT MANURE

Bat manure is the dry excrement of bats.

5. BAT GUANO

Bat guano is partially decomposed bat manure.

First Reading as Tentative

ANALYSIS

The word *analysis*, as appiled to fertilizer, shall designate the percentage composition of the product expressed in those terms that the law requires and permits.

Proposed Definitions

1. CALCIUM NITRATE

Calcium nitrate (*nitrate of lime*) is a commercial product consisting chiefly of calcium nitrate, and it should contain not less than fifteen per cent (15%) of nitrogen.

2. SUPERPHOSPHATE

Superphosphate is a commercial product, the phosphoric acid (P_2O_5) content of which is chiefly in the form of mono-calcium phosphate, and containing variant proportions of calcium sulfate.

Superphosphate is a commercial product, the phosphoric acid (P_2O_5) content of which is chiefly in the form of mono-calcium phosphate. If it contains 23 per cent (23%) or less of available phosphoric acid, it should have been made by the sulfuric acid process; if it contains over 23 per cent (23%) of available phosphoric acid, it should have been made by the phosphoric acid method.

Ammoniated superphosphate is a product that contains available phosphoric acid (P_2O_5) as phosphates of both calcium and ammonium.

Ammoniated superphosphate is the product obtained when superphosphate is treated with ammonia.

L. S. WALKER
H. D. HASKINS
W. C. JONES
G. S. FRAPS
L. E. BOPST
W. H. MACINTIRE

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

The appreciation of the Association each year is due the referees and collaborators, whose untiring work in the development of analytical methods makes the continued progress of the Association possible. It is fitting that this appreciation be expressed in the report of this committee because of the full realization its members have of the accomplishments of these workers through the consideration of their reports. As is customary, the reports have been assigned and acted upon by the respective subcommittees whose reports are appended, with the exception of the report by the Referee on Moisture in Feeding Stuffs, H. A. Halvorson. The recommendations are so broad as to concern the work of each subcommittee, and accordingly they were given consideration by the whole Committee on Recommendations of Referees.

At the last meeting it was recommended and approved by the Association that a committee be appointed to study the whole moisture question with the object of selecting one official vacuum method for all materials and so far as possible, correlating the other moisture methods, and that the Chairman of the Committee on Moisture be the Associate Referee on Moisture, *This Journal*, **19**, 47 (1936).

After making a comprehensive review of the moisture methods the Committee concluded that there is much unnecessary duplication in procedures arising out of differences in one or another detail, some important and some apparently minor in importance; discrepancies in the status of methods; and a multiplicity of methods, which apparently can be reduced. Although the Committee realizes the impossibility of perfecting a single procedure suitable to fit the conditions met with in all types of products, it concluded that products may perhaps be classified according to their most suitable moisture method, and that the present methods may be simplified. To accomplish these ends the referee recommends—

(1) That all proposed changes in the moisture methods of this Association be first considered and approved by the Committee on Moisture.

(2) That the committee be given the privilege of assigning work to additional investigators, who shall report directly to the committee.

(3) That the committee and the associate referees on moisture continue the work of correlating the moisture methods of the Association.

Without any doubt the objectives to be attained are desirable, but a consideration of the best procedure to follow is one on which your Committee has been engaged.

The approach to the problem of perfecting methods by way of studies directed to the constitutents rather than to specific products is not new to the Association, as evidenced by former studies on this same constituent, moisture. Following the presentation of a paper by Trowbridge on "Water Determination Without the Aid of Heat" in 1908 (Bur. Chem. Bull. **122**, p. 219), proposing drying in a vacuum desiccator over sulfuric acid, at room temperature, a referee on water in foods was appointed and attempts were made to establish the applicability of this method to numerous foods. Although the method became official and the refereeship enlarged to include feeding stuffs, it was never demonstrated that it could be universally applied. In fact, several workers showed that with some foods it was inaccurate. The work continued for a number of years, and in 1919 Clarke, as referee (*This Journal*, **4**, 344), pointed out an unimproved situation in respect to uniformity of moisture methods after the ten intervening years of study. Clark's was the last report on the subject of moisture, but in the meantime the individual referees had studied moisture methods for many specific commodities, and they had been adopted. While no definite action is recorded, it is apparent that the Association has found it more expedient to study the application of methods

as the characteristics of the product demand rather than to attempt to so limit the methods that there may be doubt as to their applicability to some products. Unison in methods, while desirable, is not so important as comparability of results.

Your Committee feels that simplification and unification of procedures, as adapted to as many different products as possible, are to be desired and are applicable not only to moisture but equally so to many other constituents. It believes, however, that this end should be accomplished by action of referees now provided, through recommendation to the constituted authority, the Committee on Recommendations of Referees. Our failure to progress toward the desired goal is due perhaps to the interest of the individual referee in his own subject to the exclusion of any consideration of similar problems of others, and to a belief that study of present problems on new methods is more important than studies on methods of long standing, in the limited time most referees have to devote to our work. Because of these natural circumstances, we must realize that a continuation along present lines will not likely bring about any of the admittedly desirable objectives. This Committee believes that steps in the direction of standardization of methods should be begun on methods for moisture, as they offer the opportunity to determine whether or not the plan is feasible. If it is, the procedure can be similarly applied to other constituent determinations. Let us not overlook the need for rapid, accurate methods suitable to a limited number of products. Since it is also considered that a committee will be helpful in furthering the work, it is accordingly recommended—

(1) That a committee of three on moisture in foods and feeding stuffs be appointed to inaugurate studies on moisture methods through the respective referees.

(2) That this committee suggest for study if necessary, the methods that in their opinion offer the best prospects of applicability to the largest group of products.

(3) That the committee assist in the accomplishment of the program by acting as a clearing house in correlating the details of the procedures studied by each referee.

(4) That the committee add to the recommendations of the referees any additional ones regarded as fitting.

It is not expected that the studies on moisture will curtail the work planned on other methods.

With the completion of the fourth edition of *Methods of Analysis* the Editorial Committee on Methods of Analysis has asked each referee to review the chapter for which he is responsible and to recommend that the necessary changes be made and included in a compilation of errata and referee emendations. However, it has not been possible to place a copy of the book in the hands of each referee in time for this meeting, and provision should be made for including such changes by action of the Committee on Recommendations of Referees. It is therefore recommended

that the Committee on Recommendations of Referees be authorized to include in the errata and referee emendation sheet such changes as are necessary to correct obvious errors brought to the committee's attention after the meeting and before the publication of the sheet.

H. A. LEPPER

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By H. R. KRAYBILL (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Chairman;* G. L. BIDWELL and G. E. GRATTAN

STANDARD SOLUTIONS

It is recommended—

(1) That studies of methods of direct standardization of acid solutions be continued.

(2) That standard solutions of oxidation and reduction be studied.

(3) That the tentative methods for the preparation and standardization of solutions (p. 681) be studied collaboratively with a view to their adoption as official methods.

INSECTICIDES, FUNGICIDES AND CAUSTIC POISONS

It is recommended–

(1) That the study of methods for the determination of the pyrethrins in pyrethrum powder be continued.

(2) That the study of methods for the analysis of derris and cubé preparations be continued.

(3) That methods for the determination of naphthalene in poultry lice products be studied.

(4) That the Willard-Winter method for the determination of fluorine, pp. 46, 47, 19 (b) and (c), be modified by the addition of the following warning: "Not applicable in the presence of aluminum silicate."

(5) That the lead chlorofluoride method for the determination of fluorine be studied next year.

(6) That further study be made of the optimum *p*H value for the titration of fluorine with thorium nitrate.

FEEDING STUFFS

It is recommended—

(1) That an associate referee be appointed to study methods for the determination of lactose in mixed feeds.

* These recommendations, submitted by Subcommittee A, were approved by the Association, and unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(2) That the work on the determination of fluorine in feeding stuffs be continued.

(3) That a survey be made of analytical methods suitable for the determination of this element in mineral supplements to feeds.

(4) That such methods, when found, be tried on fluorine in feed mixtures.

(5) That further study be given to the detection of mineral adulterants in feeds.

(6) That a study be made of methods for the detection of adulteration of condensed milk products.

(7) That a study be made of methods for the detection of adulteration of cod liver oil.

(8) That further study be given to the microanalytical detection of iodine in feeding stuffs.

(9) That the method proposed by Elmslie and Caldwell be further studied and that other simplified procedures for the determination of iodine in mineral mixed feed be considered for collaborative work.

(10) That collaborative work be done on the present tentative method for the determination of calcium oxide in feeds (p. 347, **44**), with the view to the final adoption of the method as official.

(11) That the tentative Vitamin D assay by preventive biological test (p. 351, **56**), be modified as suggested by the associate referee (see p. 72).

(12) That the modified alkaline titration method submitted by the associate referee be studied further and not adopted as official at this time as recommended by the associate referee.

(13) That the use of an indicator solution, as suggested by Bartholomew and Raby, be not made an optional part of this method, as suggested by the associate referee, but that this matter be studied further with a view to selecting one or the other of the optional procedures.

(14) That the photoelectric turbidimetric method of Bartholomew and Raby be studied collaboratively before adoption as official is recommended.

(15) That as suggested by the associate referee, the qualitative test for cyanogenetic glucosides in feeds and similar materials (p. 347, **45**) be adopted as official (first action) and that the method be studied further with a view to adoption as official (final action).

(16) That the tentative Prussian blue method (p. 348, **48**) be deleted.

(17) That since present colorimetric methods involve considerable manipulation and have no advantages over the titration method, no further study be given to such methods.

(18) That if additional methods are desired, the turbidimetric methods of Fox or Roe, and the nesslerization method of Gale and Pensa be studied.

(19) That studies on the determination of fat in fish meal be continued.

(20) That additional studies be made on the method for vitamin B complexes recommended by the associate referee, *This Journal*, **19**, 595 (1936), before it is recommended as a tentative method.

(21) That the study of the various factors affecting bone ash determination and other criteria used for interpreting the amount of calcification in young chicks be continued.

(22) That studies be made to determine the most suitable methods for the extraction and isolation of carotene from feeding stuffs.

(23) That an attempt be made to improve the reference standard used in the colorimetric method for the determination of carotene.

(24) That studies be made of the use of photoelectric or neutral wedge photometers in the determination of carotene.

(25) That since no report was given by the Associate Referee on Qualitative Tests for Proteins, the Committee repeats the recommendation made last year, that studies of qualitative tests for proteins be continued.

FERTILIZERS

It is recommended—

(1) That the Associate Referee on Phosphoric Acid consider the matter of designating the methods for the determination of total phosphoric acid in regard to which ones are suitable for fertilizers containing cottonseed meal, specifying the kind of filter paper suitable for estimation of water-soluble phosphoric acid, stating how long the residue may stand between washings out of the water-soluble phosphoric acid, and estimating the citrate-soluble phosphoric acid.

(2) That further study be made of the nature of the citrate-insoluble components of phosphate materials with a view to improving the method of determining the availability of such materials.

(3) That the Associate Referee on Nitrogen consider the matter of examining reduced iron to see if any lots contain nitrogen, elimination of the reduced iron method for nitrogen, and cooperation with the Referee on Standard Solutions in improving the methods for volumetric solutions used in the estimation of nitrogen.

(4) That in the official method for the determination of nitrate nitrogen (p. 27, **34**), the first part of the first sentence under **(b)** be changed to read: "Weigh out 2.0 g of the fertilizer mixture on a 11 cm Whatman No. 2 filter paper, etc." (change in an official method, first action).

(5) That the method for the determination of water-insoluble nitrogen in cyanamide (p. 27, **36**) be further studied collaboratively with a view to its final adoption as official.

(6) That the Associate Referee on Potash consider the matter of providing for the use of a factor weight, elimination of the barium chloride

method, filtration of the potash solution after ignition and solution if necessary, changing the strength of the platinum chloride solution, and recommending a method for the recovery of platinum.

(7) That the problem of foaming during the boiling of the sample in the present official method for the determination of potash in mixed fertilizer (p. 30) be studied.

(8) That further study be given to the following suggested change in the last two sentences of section **44(a)**, p. 30, "Weigh and remove the chloroplatinate precipitate by washing with hot H_2O, using slight suction. Work with 80% alcohol three times, dry as before, and weigh (loss equals K_2PtCl_6). Calculate to K_2O."

(9) That studies be made of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination (p. 30, **43**).

(10) That the words, "after 15 minutes," be inserted at the beginning of the sixth sentence, line 9, under **44(a)**, p. 30, so that the sentence will read as follows: "After 15 min. filter on a Gooch crucible . . . ."

(11) That the Associate Referee on Acid-forming or Non-acid-forming Quality of Fertilizers ascertain whether any samples of phosphate rocks are acid by the method for acid-forming or non-acid-forming quality in fertilizer.

(12) That the modified methods submitted by the associate referee for the determination of the acid- and base-forming quality of fertilizer be further studied.

(13) That the present tentative method for the same determination (p. 34, **55**), be studied in regard to the following particulars: (a) substituting 0.5 *N* NaOH in the titration in place of 1.0 *N* NaOH; (b) minimizing the loss by spattering; and (c) eliminating water-soluble material coarser than 20-mesh by wet sieving, before the method is applied.

(14) That the basicity of phosphate rock and other factors that affect the method be studied further.

(15) That the title of the method adopted as tentative last year (p. 34, **54**) be changed from "Total Magnesia" to "Acid-Soluble Magnesia," and that part of the first paragraph of the method be changed to read as follows: "Weigh 2 g of sample into a 200 cc volumetric flask, add 10 cc of HCl and 30 cc of HNO_3, boil gently for 30 min., cool, and dilute to volume with H_2O. Pipet an aliquot containing not more than 30 mg of MgO into a 250 cc beaker, add 6 cc of H_2SO_4 (1+1) etc."; and that with these minor changes the method be adopted as official, first action.

(16) That the method for the determination of magnesia in water-soluble compounds used as sources of magnesium in mixed fertilizers be studied collaboratively.

(17) That the study of methods for the determination of active magnesia in mized fertilizers be continued, with emphasis on a search for

shorter procedures for determining magnesia after it is dissolved from fertilizers.

(18) That a method for the determination of acid-soluble manganese be formulated.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That collaborative study be made of the ammonium chloride distillation method for the determination of exchangeable bases and saturation capacity of soils.

(2) That the determination of *p*H values in the soils of the arid and semi-arid regions be studied and that particular attention be directed toward the study of such determination by means of the glass electrode.

(3) That the Associate Referee on *p*H Value in Soils of the Humid Regions study the adaptability of the glass electrode to such soils.

(4) That work be continued on the determination of selenium in soils.

(5) That since no report on liming materials was presented by the associate referee, the Commitee repeats the recommendation of last year that studies on liming materials be continued.

(6) That the fusion and the volatilization methods for the determination of iodine in soils (pp. 8–10) be adopted as official (first action).

(7) That since no report on fluorine or arsenic in soils was presented, the Committee recommends that these studies be continued.

PLANTS

It is recommended—

(1) That the titrimetric method for the determination of iron (p. 122, **8**) be compared with the colorimetric method (**7**) and studied collaboratively with the view to adoption as official.

(2) That collaborative work be done on the perchloric acid method for the determination of sodium and potassium (p. 126, **16**) and on the uranyl acetate method for the determination of sodium only (122, **18**) with the view to adoption as official.

(3) That studies on forms of nitrogen, carbohydrates, and less common elements in plants be continued by the associate referees.

(4) That studies of methods for the determination of copper, lead, and fluorine in plants be continued.

(5) That the Hicks method, *J. Ind. Eng. Chem.*, **5**, 650 (1913), for the determination of potassium be studied, and that an associate referee be appointed to carry on all the work on sodium and potassium mentioned in this report.

(6) That an associate referee be appointed to study methods for the determination of inulin in plants.

(7) That the following changes be made in the procedures found in the 4th edition of *Methods of Analysis, A.O.A.C.*

Page 121, par. 5, l. 3.—Change "Moisten the residue with 5 cc of HCl . . ." to "Moisten the residue with 5–10 cc of HCl, boil about 2 min. . . . ."

Page 122, par. 5(b), l. 2.—After "Moisten the residue with 5–10 cc of HCl," insert "boil for about 2 min."

Page 140, par. 59, l. 12.—Change "add 20 cc of H_2O and 10 cc of 0.5 *N* NaOH soln" to "add 20 cc of H_2O and 10 cc of NaOH soln, II, 19(g)."

(8) That since no report was given by the Associate Referee on Less Common Metals in Plants, the Committee repeats the recommendation of last year, that further collaborative study be made of the combustion method for the determination of iodine in plant material adopted as tentative in 1934 (p. 133).

(9) That study of the reagents used in the determination of chlorine for the introduction of free iodine be continued.

(10) That tentative volumetric method II for the determination of chlorine (p. 131, **38**) be studied collaboratively, and that the associate referee be advised at once when any analyst encounters difficulty with the method.

(11) That the last two sentences in the description of Reagent **(f)**, p. 132, be deleted and the following substituted: "Decant and test the soln by adding 25 cc of it to 5 cc of Reagent **(d)** and 25 cc of Reagent **(e)**. No more than a faint blue should appear after the solution has stood 5 min. If necessary, repeatedly apply fresh portions of Reagent **(e)** to the residue of iodine crystals."

(12) That the sentence, p. 132, 2nd line from the bottom of page, "When the digest is cool, add 75 cc of H_2O and cool to room temp.," be revised to read, "When the digest is cool, add 175 cc of H_2O, boil for 5–10 min., and cool to room temp."

(13) That under Reagent **(e)**, p. 132, the words "boil for 5–10 min." be inserted after the words, "to 1 liter of H_2O."

(14) That since no report was made on carbohydrates, the Committee repeats the recommendation made last year to the affect that the determination of sucrose in plants and the determination of starch in plants be further studied.

(15) That since no report was given on forms of nitrogen in plants, the Committee repeats the recommendation of last year that studies of methods for determining different forms of nitrogen be continued.

ENZYMES

That since no reports were given, the Committee repeats the recommendations made last year to the effect that the studies on catalase be continued and that the method for the assay of papain be further studied, preferably on more active preparations, including those of bromelin, and that further information regarding the best method of activating the enzyme be sought.

LIGNIN

That since no report was given, the Committee recommends that the method adopted last year as tentative for the determination of lignin in plants be studied collaboratively with a view to its adoption as official.

PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

It is recommended—

(1) That the methods for raw and boiled linseed oil be made official, final action.

(2) That a study be made of the following additional methods of testing varnishes: Abrasion resistance, hardness, skinning, and alkali resistance.

(3) That study on the accelerated weathering test of paints be continued.

(4) That if possible, collaboration with the Subcommittee on Accelerated Weathering Tests of the American Society for Testing Materials be attempted.

VITAMINS

It is recommended—

(1) That the method submitted by the associate referee for the assay of vitamin D milk be adopted as tentative (see p. 78), and that collaborative work be continued until certain options can be eliminated.

(2) That further study be made of spectroscopic methods for the determination of vitamin A.

LEATHERS AND TANNING MATERIALS

No report was presented. It is recommended that the referee conduct such studies as will keep the methods up to date.

DISINFECTANTS

No report was presented. It is recommended that further studies be made leading to the adoption of additional methods.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By A. E. Paul (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* L. B. Broughton and H. J. Fisher.

NAVAL STORES

No reports on rosin and turpentine were submitted. It is recommended that these subjects be continued.

RADIOACTIVITY

It is recommended—

(1) That studies be continued on the application of the gamma ray scope.

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* These recommendations, submitted by Subcommittee B, were approved by the Association, and unless given otherwise all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(2) That studies be undertaken on the application of the quantum counter to radioactive products.

DRUGS

MICROCHEMICAL METHODS FOR ALKALOIDS

It is recommended—

(1) That the confirmation test for theophylline be adopted tentatively in addition to the now tentative procedure (see p. 82).

(2) That the proposed method for apomorphine be adopted tentatively (see p. 79), and that the test proposed by Shupe be further studied collaboratively with a view to possible adoption as a confirmatory test.

(3) That the proposed method for hydrastine be adopted tentatively (see p. 79).

(4) That the study of anabasine be referred to the Referee on Insecticides and that it be given no further consideration as a drug.

(5) That the study of pelletierine be discontinued since the market product is a mixture of alkaloids which is not suited for microchemical study.

(6) That hydrastinine, cytisine and berberine be studied.

MICROCHEMICAL METHODS FOR SYNTHETICS

It is recommended—

(1) That the proposed methods for the determination of barbital, phenobarbital, amytal, and ethylhydrocupreine be adopted tentatively (see p. 80).

(2) That methods for the determination of acetylsalicylic acid, benzoic acid, salicylic acid, and dial be studied.

HYPOPHOSPHITES

The Bruening method, which was studied by the Associate Referee is essentially that which is now recognized in the National Formulary VI for ammonium hypophosphite. The Associate Referee's recommendation that the applicability of the method for mixtures of hypophosphites in sirups and elixirs be studied is approved.

SANTONIN, PHENOLPHTHALEIN AND CALOMEL IN TABLETS

It is recommended that the subject be continued. The results on santonin and calomel are quite satisfactory. Of the six collaborators, two reported excellent results on the phenolphthalein. This may show that experience is required with the method described in *Methods of Analysis*, (**76**, p. 569). This method is less comprehensive than that given by Palkin in his original report, *This Journal*, **7**, 14 (1923). It is suggested that the Associate Referee consider this feature next year.

BENZYL COMPOUNDS

It is recommended that the subject be discontinued for the present because the use of benzyl compounds in medicine has decreased ma-

terially. The proposed saponification method is promising, but it would require further study before adoption.

RHUBARB AND RHAPONTICUM

It is recommended that the work be continued. A satisfactory method for distinguishing between rhubarb and rhaponticum is very much needed.

HEXYLRESORCINOL

It is recommended—

(1) That the bromine method as modified by the associate referee be adopted as tentative (see p. 81).

(2) That the subject be reassigned for study of the product in mixtures.

CHEMICAL ASSAY FOR ERGOT ALKALOIDS

It is recommended that the subject be continued for additional collaborative study. The Associate Referee studied the Hampshire-Page chemical method for the separation of water-soluble and water-insoluble alkaloids of ergot. He also adapted the wedge photometer to the method.

NITROGLYCERIN IN MIXTURES

It is recommended that the topic be continued, and that the Associate Referee submit samples for collaborative tests.

GUAIACOL

It is recommended that an effort be made to develop methods for this important subject.

BIOLOGICAL TESTING

It is recommended that this subject be continued.

IODINE IN IODINE OINTMENT

It is recommended that the methods proposed by the Associate Referee for the estimation of free iodine, potassium iodide, and organically combined iodine be subjected to collaborative study.

ACETPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN

It is recommended that this topic be continued.

PYRIDIUM

It is recommended that the subject be continued.

AMINOPHYLLINE

It is recommended—

(1) That the method devised by the Associate Referee, which is applicable to tablets and solutions of theophylline, be adopted as tentative (see p. 82), although this subject is included in the U.S.P.

(2) That no further work be done on aminophylline.

GUMS IN DRUGS

It is recommended that this subject be continued.

CINCHOPHEN AND SODIUM BICARBONATE IN TABLETS

It is recommended—

(1) That the Associate Referee's method be adopted as a tentative procedure (see p. 83).

(2) That a study be made of cinchophen in the presence of salicylates.

STABILITY OF POTASSIUM IODATE VOLUMETRIC SOLUTIONS

It is recommended that this subject be discontinued. The Associate Referee studied a 0.025 *M* solution while the U.S.P. mentions the 0.05 *M* and the N.F. includes 0.05 *M* and 0.02 *M* strength. The keeping qualities of the 0.025 solution are satisfactory, no change having been found in 20 months.

DINITROPHENOL

It is recommended—

(1) That the method originally devised by Shupe and proposed by the present Associate Referee be tentatively adopted (see p. 82).

(2) That no further work be done on this subject at the present time.

THEOBROMINE CALCIUM

No report was received. It is recommended that the topic be continued.

CHLORBUTANOL

It is recommended that this subject be continued.

PHENOLPHTHALEIN AND ACETYLSALICYLIC ACID

This Association has adopted separate methods for the determination of phenolphthalein and acetylsalicylic acid. The Associate Referee's problem therefore is the complete extraction and separation. He has devised a method for this separation, and it appears to be sound. Nevertheless some unsatisfactory results were reported by the collaborators. Possibly this may be due to lack of experience with the official methods for the determination of the two ingredients. It is recommended that the method and possibly other methods for the separation be further studied.

HOMATROPINE IN TABLETS

It is recommended that the Associate Referee's method and possibly other methods be studied collaboratively next year, with special attention given to small quantities of the drug.

CUBEB

It is recommended that the subject be continued.

METHODS FOR THE TITRATION OF ALKALOIDS

It is recommended that this topic be discontinued because it is the opinion of this Committee that each alkaloid constitutes a separate problem.

NEW TOPICS

It is recommended that the following new topics be assigned to associate referees for study during the coming year: 1. Emulsions of Cod-Liver Oil, 2. Elixir of Terpin Hydrate and Codeine, 3. Aminopyrine and Phenobarbital in Mixtures, 4. Ointment of Mercuric Nitrate, 5. Daphnia Methods, and 6. Theophylline Sodium Salicylate.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By W. B. WHITE (U. S. Food and Drug Administration, Washington, D. C.), *Chairman;* J. O. CLARKE and G. G. FRARY

CANNED FOODS

It is recommended—

(1) That the method for the determination of total chlorides in tomato juice presented by the associate referee be adopted as tentative (see p. 78).

(2) That studies of methods for the analysis of tomato juice be continued.

(3) That studies of methods for quality factors and for fill of container be continued.

DAIRY PRODUCTS

It is recommended—

(1) That the tentative method for the determination of citric acid in milk (p. 264, 5) be further studied.

(2) That the methods for the determination of casein and albumin in milk be studied with a view to the adoption of not more than one method for each protein.

(3) That studies be made of alternative methods for detecting gelatin in milk and cream.

(4) That the revision of the official optical method for the determination of lactose in milk proposed by the associate referee be adopted as official, first action (see p. 70).

(5) That studies on other methods of clarification of milk for the optical determination of lactose be continued.

* These recommendations, submitted by Subcommittee C, were approved by the Association, and unless given otherwise all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(6) That the tentative method for preparation of sample of butter (p. 288, **77**) be dropped and studies discontinued. (The official method has acquired such a wide-spread use that there is no apparent need for the tentative method.)

(7) That the associate referee define more closely the conditions of time and temperature in the official method for the preparation of butter samples (p. 288, **76**).

(8) That the indirect method for the determination of fat in butter be further studied with a view to removing the last traces of fat.

(9) That the tentative method for the determination of ash in cheese (p. 291, **93**) be adopted as official (first action).

(10) That the tentative method for the determination of total chlorides in cheese (p. 291, **94**) be modified as suggested by the associate referee and adopted as official, first action (see p. 70).

(11) That the associate referee study methods of isolating fat from cheese for the determination of fat properties and constants, with special reference to such treatments as promise a minimum change in the properties of the fat. The applicability of such methods to other dairy products should be kept in mind.

(12) That the determination of fat, and the Reichert-Meissl value of the same, in malted milk be further studied.

(13) That studies of methods for the determination of casein in malted milk be continued.

(14) That studies of mounting media in the microscopic method for the identification of malted milk be continued.

(15) That methods for the determination of lactic acid in dried milk be further studied.

(16) That studies of methods for the detection of neutralizers in dairy products be continued.

(17) That methods for the detection of decomposition in dairy products be studied.

(18) That studies of methods for the detection and determination of extraneous matter in dairy products be continued.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That studies be continued on methods for the determination of acidity of the lipoids (rapid method) and identification of the acid reacting substances, ammonia nitrogen and acid-soluble phosphoric acid, and any other methods for demonstrating decomposition.

(2) That studies of methods for the determination of cholesterol and fat be continued.

(3) That studies of methods for the determination of sugar, added salt, and glycerol be continued.

(4) That studies be continued on the method for water-soluble and crude albumin nitrogen in dried eggs.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the methods presented by the referee for the examination of oysters and scallops, namely, preparation of sample, total solids, and total chlorides by the open Carius method, be adopted as tentative (see p. 70).

(2) That studies be made of the methods suggested by the referee for the examination of fish.

GUMS IN FOODS

It is recommended that the tentative method for the detection of gums in cheese (p. 295, **106**) be further studied in relation to its applicability to foods other than cheese.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the methods submitted previously by the referee for the qualitative detection of soybean flour in meat products, *This Journal*, **19**, 409 (1936), be adopted as tentative.

(2) That studies of methods for the detection of nitrate and nitrite nitrogen in meat products, including meat extracts and curing solutions, be continued.

(3) That studies of methods for the detection of dried skim milk in meat products be continued.

METALS IN FOODS

It is recommended—

(1) That studies on arsenic isolation, on ashing and other sample preparation, and on the molybdenum blue method for arsenic determination be continued.

(2) That studies on increasing the range of the Gutzeit method be continued.

(3) That studies on micro methods for the determination of copper be continued.

(4) That studies on micro methods for the determination of zinc be continued.

(5) That collaborative studies on methods for the determination of fluorine in phosphates and baking powder be continued, and that special attention be given to methods of sample preparation for organic materials.

(6) That studies of methods for the determination of lead be continued, with special reference to oils and baking powders, and to the simplification of methods for removing interfering substances.

(7) That studies on methods for the determination of selenium be continued.

(8) That studies on methods for the determination of mercury be continued.

(9) That the word "phosphate-free" be inserted after "10%" in line 1, of **13(j)**, p. 378, and also after "10 g of," in line 1, of **13(p)**, p. 379.

OILS, FATS, AND WAXES

It is recommended—

(1) That the Fitelson method for the detection and approximate determination of tea seed oil, *This Journal*, **19**, 496 (1936), be made official (first action).

(2) That the Kaufmann thiocyanogen method be further studied.

(3) That both the Malfatti and the Stout and Schuette methods for the preparation of aldehyde-free alcoholic potassium hydroxide, p. 412, ***22***, be adopted as official (second action).

(4) That collaborative studies be conducted on methods for the determination of acetyl value and hydroxyl value.

(5) That the associate referee's method for the determination of oil in flaxseed be made official, first action (see p. 74), and that studies be made on its application to other commercially important oil seeds.

(6) That methods for the determination of free fatty acids be studied.

(7) That the specifications for the titer thermometer be amended in accordance with the revised Bureau of Standards specifications (see p. 75), and adopted as official (second action).

SPICES AND CONDIMENTS

It is recommended—

(1) That studies on the method for the assay of ginger be discontinued.

(2) That studies be made of the application of assay methods to other spices.

(3) That methods for determination of total ash in vinegar be studied, collaboratively with particular attention given to ashing temperature, desiccation, weighing, and the use of suitable covers for the ashing dishes, and that study of the use of sucrose or other substances for reducing the time of ashing be discontinued. (The Committee suggests study of Method **58(a)**, p. 456, with a view to defining more closely temperature and time factors.)

(4) That inasmuch as the determinations for soluble and insoluble phosphoric acid in vinegar have been demonstrated as empirical, studies be continued with a view to standardizing the directions.

(5) That the official method for the determination of solids be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

(6) That methods for the detection of caramel in vinegar be studied.

MICROBIOLOGICAL METHODS FOR CANNED FOODS

It is recommended that the various studies on microbiological methods of a general character be discontinued, and that studies be undertaken on methods for the microbiological examination of the following products: Canned vegetables, canned tomatoes and canned fruits, canned fishery products, canned meats, and sugar.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Chairman;* J. A. LECLERC and W. C. JONES

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That methods for determining acetyl-methyl carbinol and diacetyl in food products be studied.

(2) That methods for determining the so-called unfermentable sugars of molasses be studied.

(3) That the following note be added to the directions for determination of Canadian lead number (p. 492, **113**): "Filtration may be facilitated and the necessity of keeping the precipitate in the crucible covered with water obviated by stirring a weighed quantity (0.5 gram or less) of dry asbestos fiber with the precipitate and supernatant liquid shortly before filtration."

(4) That the study of maple flavor concentrates and imitations be continued.

(5) That the study of lead precipitate be continued and that a sufficiently large number of samples be used to permit definite conclusions to be drawn.

(6) That the study of the effect of clarifying agents upon the polarization of food products be extended to the examination of jellies and other pectin-containing materials.

(7) That studies on the determination of moisture in honey be continued.

(8) That the refractive indices of invert sugar solutions and the change in refractive indices with change of temperature be determined for such products as invert sugar solutions, table sirups, etc.

(9) That study of polariscopic methods be continued along the lines covered by the recommendations made and approved in 1931 and 1932 and continued in 1933.

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* These recommendations, submitted by Subcommittee D, were approved by the Association, and unless given otherwise all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(10) That study of chemical methods for reducing sugars be continued.

(11) That study of drying, densimetric and refractometric methods be continued.

WATERS, BRINE AND SALT

It is recommended—

(1) That the method for the determination of moisture in effervescent and mineral salts presented by the associate referee last year, *This Journal*, **19**, 376 (1936), be further studied. The designation of this method and of the other methods presented last year as tentative is an error.

(2) That an associate referee under drugs be appointed to study methods of analysis for effervescent potassium bromide with caffeine and similar preparations.

ALCOHOLIC BEVERAGES

It is recommended—

(1) That the following tentative methods for the examination of beer (p. 148) be advanced to the status of official (first action):

Sec. **2.**—Color; Sec. **4.**—Apparent Extract; Sec. **7(a), (b)** and **(c).**—Real Extract; and Sec. **22(a).**—Iodine Reaction. It is also recommended that Sec. **5(b)** be changed (see p. 69).

(2) That methods for the following determinations in beer be further studied: Extract of Original Wort, Real Degree of Fermentation, Total Acid, Reducing Sugars, Dextrin, Direct Polarization, Sulfur Dioxide, Pasteurization, and Chlorides, and that Heavy Metals, especially Fe, Cu, Pb, as well as As and F; Preservatives; and *p*H also be studied.

(3) That the following methods for the examination of malt (pp. 155–157) be made official (first action): Moisture, Extract (fine grinding); Extract (coarse grinding); and Color of Wort.

(4) That the method for the determination of moisture in flour (p. 206) be studied as to its applicability to the determination of moisture in malt adjuncts (p. 161), and that the referee confer with the Committee on Moisture in the development of the studies.

(5) That a special study be made of methods for the determination of fat that might be applicable to corn grits, brewer's rice and flakes.

(7) That in the study of the method for extract in malt adjuncts consideration be given to the suggestion to use a portion of the malt in the boiling operations.

(6) That associate referees be appointed to study the following subjects: Diastatic and proteolytic activity of malt, carbon dioxide in beer (compare method in Sec. **19** with other methods, *e.g.*, Cannissaro's), malt extract in malt, and malt adjuncts.

(8) That studies in wines on the determination of sorbitol, on the detection of added water by the cream of tartar saturation method, and on

the detection of added pomace by a methyl alcohol determination be discontinued.

(9) That the studies in wines on total sulfur and esterification by lead acetate be continued.

(10) That an associate referee be appointed to study the determination of alcohol in wines by the use of the ebullioscope.

(11) That an associate referee be appointed to study methods for the determination of volatile acidity in wine.

(12) That an associate referee be appointed to study methods for the determination of sulfur dioxide in wines.

(13) That the method for the determination of alcohol by weight, p. 163, **4(b)** be changed (see p. 69).

(14) That the method presented by Wilson for the detection of thujone in liqueurs, *This Journal*, **19**, 120 (1936), be adopted as tentative.

(15) That work on the methods for the determination of fusel oil and methyl alcohol in distilled spirits be discontinued.

(16) That an associate referee be appointed to study new methods of analysis to detect adulteration of distilled spirits.

(17) That the following new methods be studied: Volatile esters, identification of gamma-undecalactone, and benzaldehyde in cordials and liqueurs.

FOOD PRESERVATIVES

It is recommended—

(1) That further work be done on the method for the determination of saccharin in nonalcoholic beverages (p. 435, **15**) to learn whether or not it is applicable to the other food products listed in the official method (p. 434, **12**).

(2) That the Illing method for the determination of benzoate of soda, *Analyst*, **57**, 224 (1932), which was found by the Referee on Meat and Meat Products to be suitable for sausage, be studied with respect to its suitability for other food products.

(3) That further studies based on the work of Tortelli and Piazza be made on the qualitative test for saccharin (p. 434, **13**).

COLORING MATTERS IN FOODS

It is recommended—

(1) That collaborative work be continued on the quantitative estimation of Ponceau SX and Ponceau 3R.

(2) That investigational work be continued on the quantitative estimation of Sunset Yellow FCF in the presence of tartrazine.

(3) That investigational work be undertaken to separate and estimate quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

(4) That the method for extraction and identification of added color in macaroni and similar products (p. 229) be made official (final action).

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That last year's recommendations on soluble solids and effect of acids on sugar on drying, pectic acid and electrometric titration of acidity, and inactive malic acid be carried over for further study.

(2) That the colorimetric methods for the determination of lactic acid, reported at this meeting by the Associate Referee on Dried Milk, and other methods be tried on fruits and fruit products.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That further collaborative work be done on the determination of vanillin and coumarin in imitation vanillas.

(2) That a further study be made of the possibility of determining glycerol in vanilla flavors by the specific gravity method.

CACAO PRODUCTS

It is recommended—

(1) That the pectic acid method for the determination of shell described in the report of the referee be subjected to collaborative study.

(2) That methods for the detection of added lecithin be further studied.

(3) That further collaborative work be done on the crude cellulose method for the determination of shell given in the associate referee's report of 1934, *This Journal*, **18**, 431 (1935).

BAKING POWDERS AND BAKING CHEMICALS

It is recommended—

(1) That the Hartmann methods for the determination of tartaric acid and tartaric radical, *This Journal*, **13**, 385 (1930), be studied collaboratively.

(2) That the methods for the determination of fluorine and lead in foods be studied in regard to their applicability to baking powder and baking chemicals.

CEREAL FOODS

It is recommended—

(1) That an associate referee be appointed to study each of the following subjects: (a) Soya flour in foods (qualitative and quantitative determination); (b) macaroni (kind of raw material used in its manufacture); (c) whole wheat flour (scalped or real); and (d) phosphated flour (original ash content).

(2) That the method for the extraction and identification of added color in macaroni products, p. 229, **79**, be adopted as official (final action).

(3) That further studies be made of methods for the determination of salt-free ash in macaroni products and baked products.

(4) That the methods presented by Munsey, *This Journal*, **18**, 497 (1935), for the determination of chlorine bleach be studied collaboratively.

(5) That further study be made of methods of extraction and colorimetric determination of alkaloids and other constituents of ergot as a means of determining ergot in rye flour.

(6) That the method for the determination of catalase in flour be further studied, and that the method for the determination of protease in flour be studied collaboratively.

(7) That the magnesium acetate method for ashing cereal products (Cereal Lab. Methods, 3rd Ed., p. 31), as modified by the associate referee, be adopted as a tentative method (see p. 69), and that study of this method be continued.

(8) That the modified method for the determination of CO_2 in self-rising flour be given further study with a view to the determination of the variables affecting the CO_2 recovery.

(9) That the method for the detection of benzoyl peroxide bleach in flour be further studied.

(10) That collaborative work be continued on the determination of color in flour.

(11) That the title of the method for determination of "viscosity" of flour (p. 221, **47**) be changed to read "Apparent viscosity," and the method be adopted as official (first action).

(12) That (a) the citric acid method (p. 224, **55**); (b) the fat method outlined by the associate referee; and (c) the lactose method, *Cereal Chem.*, **13**, 541 (1936) and *Ind. Eng. Chem. Anal. Ed.*, **8**, 298 (1936), for the determination of milk solids in bread, be continued with a view to collaborative work if the facts warrant.

(13) That further collaborative study be made of the tentative method (p. 213, **31**) for the determination of starch in flour in regard to its applicability to products other than flour.

(14) That further collaborative study be made on the Mannich and Lenz method, *J. Nat. Research Council, Canada*, **11**, 751 (1934), for the determination of starch, provided the frothing and filtering difficulties can be eliminated.

(15) That further study be made of the application of the Sullivan method for the determination of starch to food products such as bread, cake, crackers, etc., as well as cereals.

(16) That the method for the determination of H-ion concentration described in the report of the associate referee be subjected to further study.

(17) That further direct studies on the determination of unsaponifiable constituents of flour, baked and macaroni products, be discontinued.

(18) That the development of methods for the determination of the sterol content of cereals, if such data become necessary to the evaluation of the egg content of farinaceous products, be carried on in conjunction with the same work on eggs.

(19) That the subject of cold water extract flour be further studied.

MICROCHEMICAL METHODS

It is recommended that the referee subject to collaborative study methods for which there appears to be need.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-SECOND ANNUAL MEETING, NOVEMBER 29, DECEMBER 1 AND 2, 1936.[1]

I. SOILS

(1) The fusion and volatilization methods for the determination of iodine in soils (pp. 8, 9, and 10) were adopted as official (first action).

II. FERTILIZERS

(1) The first part of the first sentence of section **34(b)** of the official method for the determination of nitrate nitrogen (p. 27), was changed to the following (first action): "Weigh out 2.0 g of the fertilizer mixture on a 11 cm Whatman No. 2 filter paper, etc."

(2) After the title had been changed from "Total Magnesia" to "Acid-soluble Magnesia" (p. 34, **54**), and the first two sentences had been changed to the following: "Weigh 2 g of sample into a 200 cc volumetric flask, add 10 cc of HCl and 30 cc of HNO_3, boil gently for 30 min., cool, and dilute to volume with H_2O. Pipet an aliquot containing not more than 30 mg of MgO into a 250 cc beaker, add 6 cc of H_2SO_4 (1+1), remove the cover, and evaporate until white fumes appear," the method was adopted as official (first action).

(3) The official method for the determination of potash in mixed fertilizers, p. 30, **44(a)**, was changed by inserting in the 9th line the words "After 15 minutes," making the sentence read as follows: "After 15 min. filter on a Gooch crucible . . . . . " (First action.)

III. SEWAGE*

IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

V. AGRICULTURAL DUST*

VI. INSECTICIDES AND FUNGICIDES

The Willard-Winter method for the determination of fluorine, pp. 46, 47, **19(b)** and **(c)**, was modified by the addition of the following warning, "Not applicable in the presence of aluminum silicate."

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[1] Compiled by Marian E. Lapp, *Associate Editor*. Unless otherwise given, all references in this report are to *Methods of Analysis*, A.O.A.C., 1935, and the methods are edited to conform to the style used in that publication.

* Subject for future study

VII. CAUSTIC POISONS

No additions, deletions, or other changes.

VIII. NAVAL STORES

No additions, deletions, or other changes.

IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

The methods for raw and boiled linseed oil (pp. 107 and 110) were adopted as official (final action).

X. LEATHERS

No additions, deletions, or other changes.

XI. TANNING MATERIALS

No additions, deletions, or other changes.

XII. PLANTS

The following changes were made in the methods:

(1) In line 3 of **5**, p. 121, the direction, "Moisten the residue with 5 cc of HCl . . . ," was changed to read, "Moisten the residue with 5–10 cc of HCl, boil about 2 min. . . . ."

(2) In line 2 of **5(b)**, p. 122, the direction, "boil for about 2 min.," was inserted after the direction to "Moisten the residue with 5–10 cc of HCl."

(3) In line 12 of **59**, p. 140, the direction, "Add 20 cc of H_2O and 10 cc of 0.5 *N* NaOH soln" was changed to read, "Add 20 cc of H_2O and 10 cc of NaOH soln, **II, 19(g)**."

(4) The last two sentences of **(f)**, p. 132, were changed to read as follows: "Decant and test the soln by adding 25 cc of it to 5 cc of Reagent **(d)** and 25 cc of Reagent **(e)**. No more than a faint blue should appear after the soln has stood 5 min. If necessary, repeatedly apply fresh portions of Reagent **(e)** to the residue of iodine crystals."

(5) In Reagent **(e)**, p. 132, the words "boil for 5–10 min.," were inserted after the words "to 1 liter of H_2O."

(6) The sentence "When the digest is cool, etc.," p. 132, second line from the bottom of page, was revised to read as follows: "When the digest is cool, add 175 cc of H_2O, boil 5–10 min., and cool to room temp."

XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

XIV. MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

(1) The following methods for the examination of beer were adopted as official (first action): Color (p. 148, **2**); Apparent Extract (p. 148, **4**); Real Extract (p. 149, **7(a)**, **(b)**, and **(c)**); and Iodine Reaction (p. 152, **22(a)**).

(2) Sec. **5(b)**, p. 149, was corrected in line 2 as follows: "and divide by the sp. gr." was changed to read, "multiply by the sp. gr. of the distillate, and divide by the sp. gr. of the sample."

(3) The following methods for the examination of malt were adopted as official (first action): Moisture (p. 154, **38**); Extract (fine grinding) (p. 154, **41**); Extract (coarse grinding); and Color of Wort (p. 158, **45**).

XV. WINES

Sec. 4(b), line 2, p. 163, was corrected as follows: "and divide by the sp. gr." was changed to read, "multiply by the sp. gr. of the distillate and divide by the sp. gr. of the sample."

XVI. DISTILLED LIQUORS

The method submitted by Wilson for the detection of thujone in liqueurs, *This Journal*, **19**, 120 (1936), was adopted as tentative.

XVII. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

XVIII. COFFEE AND TEA

No additions, deletions, or other changes.

XIX. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or other changes.

XX. CEREAL FOODS

(1) The following magnesium acetate method for ashing cereal products was adopted as tentative:

REAGENT

Magnesium acetate soln.—Dissolve 4.054 g of $Mg(C_2H_3O_2)_2.4H_2O$ in 50 cc of H_2O and make up to 1 liter with 95% alcohol.

DETERMINATION

From a buret add 5 cc of the reagent to 3–5 g of flour, bread, etc., or 10 cc to a 1 g sample of bran, wheat germ, etc. Allow the mixture to stand 1–2 min., evaporate the excess alcohol if desired, and place the sample in a muffle furnace maintained at 700°, closing the door after the flaming has ceased. When incineration is complete, place the ashing dish in a desiccator until cool, then weigh. Run a blank determination on the soln and deduct the blank from the weight of crude ash. Evaporate the blank cautiously.

(2) The word "apparent" was prefixed to the title of the method for determination of viscosity of flour (p. 221, **47**), and the method was adopted as official (first action).

(3) The following sentence: "Allow the flask to stand quietly for 15

min.," was inserted in the method for the determination of H-ion concentration, p. 209, at the end of line 3.

(4) The method for the extraction and identification of added color in macaroni products (p. 229, **79**) was adopted as official (final action).

XXI. COLORING MATTERS IN FOODS

The method for the extraction and identification of added color in macaroni and similar products (p. 229, **79**) was adopted as official (final action). Similar action was recommended by the Referee on Cereals.

XXII. DAIRY PRODUCTS

(1) The official optical method for the determination of lactose in milk (pp. 266–7), was revised as follows (first action): In **15(a)**, line 2, the words "an equal" were changed to "a fivefold"; in **16**, p. 267, line 1, "1 cc" was changed to "18–20 cc"; and after the word "soln" were added the words, "followed by enough 5% phosphotungstic acid soln to make to the mark."

(2) The tentative method for the preparation of sample of butter (p. 288, **77**) was dropped.

(3) The tentative method for the determination of ash in cheese (p. 291, **93**) was adopted as an official method (first action).

(4) The tentative method for the determination of total chlorides in cheese (p. 291, **94**) was modified as follows, as suggested by the associate referee, and adopted as an official method (first action): In line 5, the sentence "Dilute etc." was changed to "Cool; filter the soln into a 200 cc graduated flask, washing the filter paper thoroly with H_2O at approximately 20°; and make to volume." In line 6, the word "soln" was substituted for "filtrate."

XXIII. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

XXIV. FISH AND OTHER MARINE PRODUCTS

The following methods for the examination of oysters and scallops were adopted as tentative:

(1) PRELIMINARY TREATMENT AND PREPARATION OF SAMPLE

APPARATUS

(a) *Funnel.*—Made of metal, tin plated or brass, 8–10″ in diameter at the top, with a stem 3″ in diameter and about 3″ long.

(b) *Measures.*—Straight sided cylindrical, made of metal, holding exactly 1 gallon and 1 quart, and having smooth rims. The plane of the rim should be level when the measure is standing on a level surface. The diameter of the top of the gallon measure should not be more than 5¼″ or less than 4¾″, the quart measure not greater than 3¾″ or less than 3¼″. Carefully calibrate these measures with standard glass measures. For estimating volumes short of level-full, use a graduated mechanic's depth gage to measure the depth from the rim to the surface of the contents. These

depth gage readings may be tabulated against the volumes or percentage shortages as desired for each measuring vessel.

(c) *Skimmer.*—A flat-bottomed pan or tray, with 2" sides. The bottom is perforated with holes ¼" in diameter and centers 1¼" apart in a square pattern. The area of the bottom of the tray should be such that the oysters are not over one layer deep (150 sq. in. for 1 quart of oysters). The skimmer should be supported over a solid tray slightly larger to receive the liquid.

(d) *Meat chopper.*—With plate with holes ⅛" in diameter. It should not leak around the handle end.

(e) *Table fork.*—With sharp-edged tines ⅛" apart (stainless steel).

(f) *Small sharp knife.*

(g) *Malted milk stirrer.*—Electric.

Shell Oysters.—Wash the shells in potable H_2O to remove all loose silt and dirt, and drain well. Shuck enough oysters to yield at least 1 pint of drained meats, into a clean dry container. Transfer the oysters to a skimmer and rinse lightly with a spray of H_2O to remove silt particles; drain 1 min. on the skimmer, and remove to a glass fruit jar or other suitable container. (Sample should be examined on the skimmer, and any pieces of shell picked out.) Prepare the sample as directed under "Procedure."

Shucked Oysters.—"Fluff" the entire contents of the commercial container, or container in which the sample is received (1 gallon or less in size) by pouring into a standard measuring vessel thru a distance of at least 1 foot, then likewise back into the container, and again into the measuring vessel. Measure the headspace with the depth gage, and determine the volume. Transfer the oysters to the skimmer, drain 1 min., return the meats to the measuring vessel, and measure the headspace. The loss in volume is the free liquid. If less than 10%, again mix in with the meats. Prepare the sample as directed under "Procedure." If the free liquid is more than 10% of the total volume, allow it to set in a tall container, remove the scum from the top, pour off the clear liquid from the shell and other sediment (do not filter), and analyze separately.

PROCEDURE

Grind the meats or the mixed meats and liquid in the meat chopper, remove any muscle that is retained inside the chopper, and comminute with the fork and knife. (For good checks it is important to cut these pieces into small bits.) Mix all together in a tin can of suitable size and stir with the electric stirrer for 5 min., keeping the entire contents of the container in motion. If the impeller spins in one spot, without moving the entire contents, raise or lower the tin container (do not filter). Keep the prepared samples under refrigeration between 1 and 10°.

NOTE: The malted milk stirrer cannot be used successfully with ground scallop meats; they should be mixed thoroly by stirring with a spatula.

2. TOTAL SOLIDS

Make duplicate determinations. Weigh quickly 10 g of the meats, liquid, or mixed meats and liquid in a flat-bottomed metal dish about 9 cm in diameter. Spread the sample evenly over the bottom of the dish. Evaporate just to dryness on a steam bath and dry for 4 hours in a water oven at a temp. between 98 and 100°. Cool in a desiccator and weigh promptly.

3. CHLORINE AS SODIUM CHLORIDE

I. *Open Carius Method*

REAGENTS

(a) *Silver nitrate soln.*—0.1 *N*. Standardize against 0.1 *N* NaCl soln containing 5.846 g of pure dry NaCl per liter.

(b) *Ammonium thiocyanate soln.*—Standardize against the 0.1 *N* $AgNO_3$ soln.
(c) *Ferric indicator.*—A saturated soln of ferric ammonium alum.

DETERMINATION

Put 10 g of the meats, liquid, or mixed meats and liquid into a 250 cc Erlenmeyer flask or beaker. Add a known volume of the $AgNO_3$ soln., more than sufficient to precipitate all the Cl as AgCl (10 cc = 0.58%) and then add 20 cc of HNO_3. Boil gently on a hot plate or sand bath until all solid matter except the AgCl is dissolved (15 min. is usually sufficient). Cool, add 50 cc of H_2O and 5 cc of indicator, and titrate the excess of silver with the thiocyanate until a permanent light brown color appears. Subtract the cc of 0.1 *N* thiocyanate used from the cc of 0.1 *N* $AgNO_3$ added and calculate the quantity of Cl as NaCl. With a 10 g sample each cc of 0.1 *N* $AgNO_3$ = 0.058% NaCl.

II. *Ashing Method with Calcium Acetate as Fixative*

To 10 g of ground meats or liquid in a Pt dish, add 10 cc of 10% Ca acetate soln and thoroly incorporate with the ground meats or liquid. Dry on the steam bath, and ash in a muffle at the lowest visible red heat (550°). (Complete ashing is not necessary.) Dissolve the ash in the Pt dish in 25 cc of HNO_3 (1+3). Add to the HNO_3 soln a known volume of 0.1 *N* $AgNO_3$ soln, more than sufficient to precipitate the chlorides as AgCl. Heat to boiling, cool, and add 5 cc of ferric ammonium sulfate indicator and titrate the excess Ag with the thiocyanate until a permanent light brown color appears. From the number of cc of 0.1 *N* $AgNO_3$ used, calculate the quantity of Cl as NaCl. With a 10 g sample, each cc of 0.1 *N* $AgNO_3$ soln = 0.058% NaCl. (Considerable chlorine is lost on ashing without a fixative.) Make a correction for Cl in the 10 cc of 10% Ca acetate soln if it is not free from chlorides.

XXV. FLAVORING EXTRACTS

No additions, deletions, or other changes.

XXVI. FRUITS AND FRUIT PRODUCTS

No additions, deletions, or other changes.

XXVII. GRAIN AND STOCK FEEDS

(1) The method for vitamin D assay by preventive biological test (p. 351) was modified by the associate referee to read as follows:

VITAMIN D ASSAY BY PREVENTIVE BIOLOGICAL TEST

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing the vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

BASAL RACHITIC RATION

	per cent
Ground yellow corn	59
Pure wheat flour middlings	25
Crude domestic acid precipitated casein	12
Calcium carbonate (precipitated)	1
Calcium phosphate (precipitated)	1
Iodized salt (0.02% potassium iodide)	1
Non-irradiated yeast (7% minimum nitrogen)	1

PROCEDURE

Place groups of 10 or more 1-day-old white leghorn chickens in screen-bottomed biological cages or battery brooders out of direct sunlight. (Red electric light bulbs are satisfactory as a source of heat for the cages.) Reserve one group for negative control purposes, one or more groups for positive control purposes, and one or more additional groups for each material to be assayed. Keep distilled H_2O before the chicks at all times.

Prepare sufficient basal rachitic ration for the entire feeding period (60 lbs. per 100 birds is ample). Prepare the supplemented rations at 8–12 day periods. Supplement the basal rachitic ration with corn oil in a quantity equal to the maximum addition of the oil to be assayed. (This is the ration to be fed to the negative control group.) Supplement the basal ration with one or more levels of an assayed cod liver oil, the level or levels to be so selected that the birds of one group will produce normal calcification. (These are the rations to be fed to the positive control group or groups.) Add corn oil to bring the percentage of oil up to that added to the negative control ration. (These are the rations to be fed to the other groups.)

On the second day give the groups two 15 min. feedings of their respective rations. Beginning the third day, feed the rations *ad libitum* for 21 days.

Kill the birds, remove the left tibia of each bird, and clear of adhering tissue. (To facilitate removal of adhering tissue, the bones may be placed in boiling H_2O for not more than 2 min.) If composite ashing of the tibiae of the groups is to be made, preserve the opposite tibiae in ethyl alcohol. Number the bones and place in 95% ethyl alcohol. Crush, wrap in filter paper, and extract the bones for 20 hours with hot 95% ethyl alcohol, followed by 20 hours with ethyl ether. (Other solvents may be used for this fat extraction.) Dry in a moisture oven, and store in a desiccator. Determine the percentage of ash of the moisture and fat-free bones by igniting individually or by group composite in a muffle furnace at approximately 850° for 1 hour.

To obtain vitamin D potency of a product in terms of A.O.A.C. chick units, use the U.S. Pharmacopoeia "Reference Cod Liver Oil" in control rations at properly selected levels and compare the resulting control ash averages with the average produced by the material under assay. One A.O.A.C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U.S.P. Reference Cod Liver Oil in this method of assay.

(2) The qualitative test for the determination of cyanogenetic glucosides in feeds and similar materials (p. 347, **45**) was adopted as official (first action).

(3) The tentative Prussian blue method for the determination of hydrocyanic acid formed by the hydrolysis of glucosides in beans (p. 348, **48**) was deleted.

XXVIII. MEAT AND MEAT PRODUCTS

The methods submitted in 1935 by the referee, for the qualitative detection of soybean flour in meat products, *This Journal*, **19**, 409 (1936), were adopted as tentative.

XXIX. METALS IN FOODS

In the directions for preparing ammonia-cyanide mixture, **13(j)**, line 1, p. **378**, the word "phosphate-free" was inserted after "10%," and in the

directions for preparing ammonia-cyanide-citric soln, **13(p)**, line 1, p. 379, the same word was inserted after the expression "10 g of."

XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

XXXI. OILS, FATS, AND WAXES

(1) The Fitelson colorimetric method for the detection and approximate determination of tea seed oil in olive oil, *This Journal*, **19**, 496 (1936), was adopted as official (first action).

(2) The procedures of Malfatti and of Stout and Schuette for the preparation of *Alcoholic potassium hydroxide soln*, in the saponification number method, p. 412, **22**, was adopted as official (second action).

(3) The following method submitted by the associate referee for the determination of oil in flaxseed was adopted as official (first action):

OIL IN FLAXSEED

Refractometric Method

PREPARATION OF STANDARD SOLVENT

Prepare a mixture of approximately 74% halowax and 26% α-bromonaphthalene by weight, and carefully adjust the composition of the mixture to a refractive index of 1.63940 at 25.0°. (If a temperature-regulating device is available the determination of refractive index is simplified by passing H_2O at exactly 25.0° thru the water jacket of the refractometer. Equally satisfactory results may be obtained, however, by using H_2O at room temp. and making the necessary correction. For the above mixture this correction in refractive index is 0.00045 per 1°, to be added to the reading if the temp. is above 25.0° and subtracted if the temp. is below that point. It is important that all water-jacket temp. readings be made to the nearest 0.1°.)

Keep the soln in a glass or lead-stoppered dark bottle and away from direct sunlight. (The refractive index of this soln should keep constant for a long period of time, but it is advisable to check it from time to time.)

PREPARATION OF SAMPLE

Obtain a representative sample of about 25 g of the clean seed either by hand quartering the original sample or by use of a mechanical sampling device. Grind the material to such a degree of fineness that after extraction with ether 95% of the sample will pass thru a 40-mesh sieve. (A motor-driven experimental roller flouring mill with 6×6 inch rolls, 40 corrugations to the inch, has been found satisfactory. The rolls should have a speed differential of 9:7 and the faster roll should have a speed of approximately 900 r.p.m. Such a mill is illustrated in U. S. Dept. Agr. Tech. Bull. 554.)

DETERMINATION

Weigh out accurately 2.5 g of the finely ground, well-mixed sample and transfer into a clean, 3 inch porcelain mortar that has been previously heated to approximately 70° in an oven or on an electric hot plate at low heat. Add approximately 1 g of reagent quality sea sand or similar abrasive and exactly 5 cc of the standard mixture of halowax and α-bromonaphthalene. (Since this mixture has a high specific gravity it is important to measure its volume accurately. This is best accomplished

with an accurately calibrated 5 cc pipet having a delivery time of not less than 15 seconds.)

Grind the mixture in the mortar vigorously for 3 min., constantly scraping into the bottom the particles of meal that are thrown against the sides of the mortar. Filter the mixture into a test tube thru a Schleicher and Schull No. 588 folded filter paper, or other fat-free paper that will yield a clear filtrate.

When the filtrate has cooled to room temp., determine its refractive index at 25.0° to an accuracy of ±0.00002. (A dipping-type refractometer equipped with interchangeable, water-jacketed, double prism heads is recommended. Such an instrument is shown in U. S. Dept. Agr. Tech. Bull. 554.) If the reading is made at a temp. other than 25.0°, make a correction as directed in the instructions for the preparation of the standard solvent, using a temp. coefficient of 0.00042 per 1°. Using Table 1, p. 76. note the percentage of oil corresponding to the refractive index of the filtrate. This is the *uncorrected value* for oil content.

Place about 2 g of the ground sample in a fine paper filter in a glass funnel and pour over it about 15 cc of petroleum ether, collecting the clear filtrate in a small shallow evaporating dish. Carefully evaporate off the ether on a steam bath or hot plate at low heat, and place the dish in an oven at 105° for 20 min. Cool the oil thus prepared to room temp. (If preferred, this sample of oil may be prepared by pressing a small sample of the ground seed in a laboratory hydraulic press and filtering the oil so obtained if it is not entirely clear.) Determine the refractive index of the oil at 25.0°. The temp. coefficient for the pure oil is 0.000357 per 1.0°, to be added if the temp. at which the reading is taken is above 25.0° and subtracted if below that temp.

From the refractive index value of the oil subtract the value 1.47780 (the refractive index at 25.0° of the composite sample of oil used in obtaining the data for Table 1). Using this difference, determine from Table 2, p. 77, the correction to be applied to the uncorrected value for oil content as determined above. If the difference is positive, add the correction; if negative, subtract.

(4) The specifications for the titer test thermometer (p. 408, **15**) were amended as follows and adopted as official (second action): Under *Range and subdivision*, "62" was changed to "66"; under *Total length*, "350–360 mm" was changed to "370–380 mm"; "*Distance to 62° mark etc.*" was changed to "*Distance to 60° mark etc.*"; under *Filling above mercury*, "Nitrogen or other suitable gas," was changed to "Nitrogen or other suitable gas, or vacuum," under *Graduation*, "at every 2° mark," was changed to read, "at each multiple of 2°"; and under *Case*, "62°C. was changed to 65°C."

XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

No additions, deletions, or other changes.

XXXIII. SPICES AND OTHER CONDIMENTS

No additions, deletions, or other changes.

XXXIV. SUGARS AND SUGAR PRODUCTS

The following note was added to the method for the determination of Canadian lead value (p. 492, **113**): "Filtration may be facilitated and the necessity of keeping the precipitate in the crucible covered with H_2O

TABLE 1.—*Conversion table for determining the percentage of oil in flaxseed from the refractive index of the halowax α-bromonaphthalene extract at 25°.*

n_D^{25}	% OIL	n_D^{25}	% OIL	n_D^{25}	% OIL	n_D^{25}	% OIL
1.61837	28.0	1.61554	32.5	1.61279	37.0	1.61012	41.5
1.61831	.1	1.61548	.6	1.61273	.1	1.61006	.6
1.61824	.2	1.61542	.7	1.61267	.2	1.61000	.7
1.61818	.3	1.61535	.8	1.61261	.3	1.60995	.8
1.61811	.4	1.61529	.9	1.61255	.4	1.60989	.9
1.61805	.5	1.61523	33.0	1.61249	.5	1.60983	42.0
1.61799	.6	1.61517	.1	1.61243	.6	1.60977	.1
1.61792	.7	1.61511	.2	1.61237	.7	1.60971	.2
1.61786	.8	1 61504	.3	1.61231	.8	1.60966	.3
1.61779	.9	1.61498	.4	1.61225	.9	1.60960	.4
1.61773	29.0	1 61492	.5	1.61219	38.0	1.60954	.5
1.61767	.1	1.61486	.6	1.61213	.1	1.60948	.6
1.61760	.2	1.61480	.7	1.61207	.2	1.60942	.7
1.61754	.3	1.61473	.8	1.61201	.3	1.60937	.8
1.61748	.4	1.61467	.9	1.61195	.4	1.60931	.9
1.61742	.5	1.61461	34.0	1.61189	.5	1.60925	43.0
1.61735	.6	1.61455	.1	1.61183	.6	1.60919	.1
1.61729	.7	1.61449	.2	1.61177	.7	1.60913	.2
1.61723	.8	1.61443	.3	1.61171	.8	1.60908	.3
1.61716	.9	1.61437	.4	1.61165	.9	1.60902	.4
1.61710	30.0	1.61431	.5	1.61159	39.0	1.60896	.5
1.61704	.1	1.61424	.6	1.61153	.1	1.60890	.6
1.61697	.2	1.61418	.7	1.61147	.2	1.60884	.7
1.61691	.3	1.61412	.8	1.61141	.3	1.60879	.8
1.61685	.4	1.61406	.9	1.61135	.4	1.60873	.9
1.61679	.5	1.61400	35.0	1.61130	.5	1.60867	44.0
1.61672	.6	1.61394	.1	1.61124	.6	1.60861	.1
1.61666	.7	1.61388	.2	1.61118	.7	1.60856	.2
1.61660	.8	1.61382	.3	1.61112	.8	1.60850	.3
1.61653	.9	1.61376	.4	1.61106	.9	1.60844	.4
1.61647	31.0	1.61370	.5	1.61100	40.0	1.60839	.5
1.61641	.1	1.61363	.6	1.61094	.1	1.60833	.6
1.61635	.2	1.61357	.7	1.61088	.2	1.60827	.7
1.61628	.3	1.61351	.8	1.61082	.3	1.60821	.8
1.61622	.4	1.61345	.9	1.61076	.4	1.60816	.9
1.61616	.5	1.61339	36.0	1.61071	.5	1.60810	45.0
1.61610	.6	1.61333	.1	1.61065	.6	1.60804	.1
1.61604	.7	1.61327	.2	1.61059	.7	1.60799	.2
1.61597	.8	1.61321	.3	1.61053	.8	1.60793	.3
1.61591	.9	1.61315	.4	1.61047	.9	1.60787	.4
1.61585	32.0	1.61309	.5	1.61041	41.0	1.60782	.5
1.61579	.1	1.61303	.6	1.61035	.1	1.60776	.6
1.61573	.2	1.61297	.7	1.61029	.2	1.60770	.7
1.61566	.3	1.61291	.8	1.61024	.3	1.60764	.8
1.61560	.4	1.61285	.9	1.61018	.4		

TABLE 2.—*Corrections to be applied to results obtained in the analysis of flaxseed for oil content by the refractometric method.*

% OIL	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
$n_D^{25}-1.4778$*									(CORRECTIONS IN TERMS OF % OIL)												
0.0001	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.03	.03	.03	.03	.03
0.0002	.03	.03	.03	.03	.04	.04	.04	.04	.04	.04	.04	.04	.05	.05	.05	.05	.05	.05	.05	.05	.06
0.0003	.05	.05	.05	.05	.05	.06	.06	.06	.06	.06	.06	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08
0.0004	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08	.09	.09	.09	.09	.10	.10	.10	.10	.11	.11	.11
0.0005	.08	.08	.08	.09	.09	.09	.10	.10	.10	.11	.11	.11	.11	.12	.12	.12	.13	.13	.13	.14	.14
0.0006	.09	.10	.10	.10	.11	.11	.11	.12	.12	.13	.13	.13	14	.14	.15	.15	.15	.16	.16	.16	.17
0.0007	.11	.11	.12	.12	.12	.13	.13	.14	.14	15	.15	.16	.16	.17	.17	.17	.18	.18	.19	.19	.20
0.0008	.12	.13	.13	.14	.14	.15	.15	.16	.16	.17	.17	.18	.18	19	.19	.20	.20	.21	.21	.22	.22
0.0009	.14	.14	.15	.15	.16	.17	.17	18	.18	.19	.19	.20	.21	.21	.22	.22	.23	.23	.24	.25	.25
0.0010	.15	.16	.17	.17	.18	.19	.19	.20	.20	.21	.22	.22	.23	.24	.24	.25	.26	.26	.27	.27	.28
0.0011	.17	.17	.18	.19	.20	.20	.21	.22	.22	.23	.24	.25	.25	.26	.27	.27	.28	.29	.29	.30	.31
0.0012	.18	.19	.20	.21	.21	.22	.23	.24	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34
0.0013	.20	.21	.22	.22	.23	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34	.35	.36	.36
0.0014	.21	.22	.23	.24	.25	.26	.27	.28	.29	.29	.30	.31	.32	.33	.34	.53	.36	.37	.37	.38	.39
0.0015	.23	.24	.25	.26	.27	.28	.29	.30	.31	.32	.32	.33	.34	.35	.36	.37	.38	.39	.40	.41	.42
0.0016	.24	.25	.27	.28	.28	.30	.31	.32	.33	.34	.35	36	.37	38	.39	.40	.41	.42	.43	.44	.45
0.0017	.26	.27	.28	.29	.30	.31	.32	.33	.35	.36	.37	.38	.39	.40	41	.42	.43	.44	.45	.47	.48
0.0018	.28	.29	.30	.31	.32	.33	.34	.35	.37	.38	.39	.40	.41	.42	.44	.45	.46	.47	.48	.49	.50
0.0019	.29	.30	.32	.33	.34	.35	.36	.37	.39	.40	.41	.42	.44	.45	.46	.47	.48	.50	.51	.52	.53
0.0020	.31	.32	.33	.34	.36	.37	.38	.39	.41	.42	.43	.45	.46	.47	.48	.50	.51	.52	.53	.55	.56
0.0021	.32	.33	.35	.36	.37	.39	.40	.41	.43	.44	.45	.47	.48	.50	.51	.52	.54	.55	.56	.58	.59
0.0022	.34	.35	.37	.38	.39	.41	.42	.43	.45	.46	.48	.49	.50	.52	.53	.55	.56	.57	.59	.60	.62
0.0023	.35	.37	.38	.40	.41	.43	.44	.45	.47	.48	.50	.51	.53	.54	.56	.57	.59	.60	.61	.63	.64
0.0024	.37	.38	.40	.41	.43	.44	.46	.47	.49	.50	.52	.54	.55	.57	.58	.60	.61	.63	.64	.66	.67
0.0025	.38	.40	.42	.43	.45	.46	.48	.49	.51	.53	.54	.56	.57	.59	.61	.62	.64	.65	.67	.69	.70
0.0026	.40	.41	.43	.45	.46	.48	.50	.51	.53	.55	.56	.58	.60	.61	.63	.64	.66	.68	.69	.71	.73
0.0027	.41	.43	.45	.46	.48	.50	.52	.53	.55	.57	.58	.60	.62	.64	.65	.67	.69	.70	.72	.74	.76
0.0028	.43	.45	.46	.48	.50	.52	.53	.55	.57	.59	.60	.62	64	.66	.68	.69	.71	.73	.75	.77	.78
0.0029	.44	.46	.48	.50	.52	.54	.55	.57	.59	.61	.63	.65	.66	.68	.70	.72	.74	.76	.77	.79	.81
0.0030	.46	.48	.50	.52	.53	.55	.57	.59	.61	.63	.65	.67	.69	.71	.73	.74	.76	.78	.80	.82	.84

* Values to be added when ($n_D^{25}-1.4778$) is positive, subtracted when ($n_D^{25}-1.4778$) is negative

obviated by stirring a weighed quantity (0.5 g or less) of dry asbestos fiber with the precipitate and supernatant liquid shortly before filtration."

XXXV. VEGETABLES AND VEGETABLE PRODUCTS

(1) The following method for the determination of total chlorides in tomato juice was adopted as tentative.

TOTAL CHLORIDES IN TOMATO JUICE

REAGENTS

(a) *Silver nitrate, soln.*—0.1 *N*. Standardize against dried, recrystallized C.P. NaCl.

(b) *Ammonium or potassium thiocyanate.*—0.1 *N*. Adjust to exact equivalence with the $AgNO_3$ soln.

(c) *Ferric indicator.*—To a 40% soln of ferric ammonium alum add 1 or 2 drops of HNO_3.

(d) *Nitric acid.*—Concentrated, C.P.

(e) *Nitrobenzene.*

DETERMINATION

Filter the tomato juice thru a folded filter, and by means of a pipet transfer 10 cc to a 200 cc Erlenmeyer flask. Add 30–40 cc of H_2O, 10 cc of the HNO_3, and a sufficient quantity of the $AgNO_3$ soln to provide a slight excess over the NaCl present (20 cc should be sufficient). Stir well. Add a few glass beads, place a short-stemmed funnel in the neck of the flask to minimize spattering, and boil gently until all solid matter except the precipitated AgCl is in soln (10–15 min.). Cool, wash down the funnel and neck of the flask with H_2O, and add 1 cc of nitrobenzene. Stopper the flask with a clean rubber stopper, and shake vigorously. Wash down the sides of the flask and stopper with H_2O, and titrate to a permanent light brown color with 0.1 *N* thiocyanate soln.

From the difference between the quantity of 0.1 *N* $AgNO_3$ added, and the quantity of 0.1 *N* thiocyanate soln used in the back titration, calculate the quantity of chlorides present as NaCl and report as grams per 100 cc of filtered juice. 1 cc of 0.1 *N* $AgNO_3$ soln = .00585 g of NaCl.

NOTE: If desired, this determination may be made on the unfiltered juice, using either a measured or an accurately weighed quantity. If a weighed amount is used, approximately 10 g should be taken.

XXXVI. VITAMINS

The following method for the assay of Vitamin D milk was adopted as tentative:

VITAMIN D MILK

COLLECTION AND PRESERVATION OF SAMPLE

Unless the sample of milk, in the original bottle, can be delivered to the assayer immediately after collection, store it under refrigeration until delivered. Make shipment to the assayer in an iced container. After acceptance by the assayer, preserve the milk in its original homogeneous state by (a) suitable refrigeration for a period of not more than 1 week, or (b) for a period of not more than 1 month by the addition of 2 drops of 10% formalin and suitable refrigeration.

PROCEDURE

As the basic procedure follow the method of assay for vitamin D in cod liver oil described in the Pharmacopoeia of the United States XI, page 478.

Feed the calculated quantities of U.S.P. Reference Oil and of the sample of milk and vary the assay period according to the following options, but feed the U.S.P. Reference Oil and the milk sample according to the same plan.

1. Proceed according to the U.S.P. XI method.
2. Feed the supplements on the first day or in three portions on each of the first 3 days of a 10-day assay period. Kill the rats on the 11th day.
3. Feed the supplements on the first day or in equal portions on each of the first 3 or 5 days of a 7-day assay period. Kill the rats on the 8th day.
4. Feed the supplements admixed with the quantity of basal ration that will be consumed in 7 or 8 days. Feed the unsupplemented basal ration during the remainder of a 10-day assay period and kill the rats on the 11th day.
5. Feed the supplements admixed with the quantity of basal ration that will be consumed in 4 or 5 days. Feed the unsupplemented basal ration during the remainder of a 7-day assay period and kill the rats on the 8th day.

Evaporated and Dried Milks.—Evaporated or dried milk may be incorporated with the basal ration (paragraphs 4 and 5) or diluted to original volume (paragraphs 1–3).

EXPLANATORY NOTE: Experimental trials and the experience of those now conducting routine assays of vitamin D milk show that departures from the U.S.P. XI method may be made in the manner in which the milk sample and the Reference Oil are fed and in the length of the assay period.

If it is necessary to feed a relatively large quantity of milk, a single feeding, as suggested below, may not be possible, and daily feedings for a period of days are necessary.

The extent of calcification at the end of a 7-day assay period is not as marked as at the end of a 10-day period Hence it may be necessary to feed higher levels of milk and Reference Oil to obtain an adequate response at the end of a 7-day period.

In paragraphs 2 and 3 several options are given as to the number of days during which supplements may be fed. These periods have been indicated because of the experimental data already accumulated upon their use and for the sake of the greater uniformity that will prevail than if the number of days of feeding were entirely optional.

XXXVII. WATERS, BRINE AND SALT

No additions, deletions, or other changes. The designation of the methods for effervescent salts described in *This Journal*, **19**, 376, 377 (1936) as tentative is in error.

XXXVIII. RADIOACTIVITY

No additions, deletions, or other changes.

XXXIX. DRUGS

(1) The following microchemical methods for the identification of apomorphine, hydrastine and theophylline were adopted as tentative:

APOMORPHINE, HYDRASTINE AND THEOPHYLLINE

REAGENTS

(a) *Ammoniacal silver nitrate soln*—Dissolve 2 g of $AgNO_3$ in 100 cc of 5% NH_4OH (freshly prepared).

(b) *Potassium ferrocyanide soln.*—Dissolve 5 g of K_4FeCN_6 in 100 cc of H_2O (freshly prepared).

(c) *Potassium iodide soln.*—Dissolve 5 g of KI in 100 cc of H_2O.
(d) *Mercuric chloride soln.*—Dissolve 5 g of $HgCl_2$ in 100 cc of H_2O.

IDENTIFICATION

Place a drop of the alkaloidal soln on a clean glass slide, add a drop of reagent by means of a clean glass rod, and without covering examine under the microscope, using low power. A magnification of 100–150 × is suitable. Note the kind of crystals formed and compare their characteristics with a control specimen of the alkaloid in the same dilution.

Characteristics of microchemical tests for alkaloids

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Apomorphine	Potassium iodide	1:50. Small crystals, which have sharp clear cut angles like those of a diamond.
Hydrastine	One drop of 5% HCl and potassium ferrocyanide	1:100. Spheres of radiating crystals. Shake slide to start crystallization. Avoid excess reagent.
Theophylline	Ammoniacal silver nitrate	1:200. Gelatinous at first: dense spheres of dark radiating needles.
	Mercuric chloride	1:150. Spheres and double tufts of dense radiating needles.

(2) The following microchemical methods for the identification of amytal, barbital, phenobarbital and ethylhydrocupreine were adopted as tentative:

AMYTAL, BARBITAL, PHENOBARBITAL AND ETHYLHYDROCUPREINE

REAGENTS

(a) *Ammonium hydroxide soln.*—Dilute 1 cc of NH_4OH with 9 cc of H_2O.
(b) *Acetic acid soln.*—Dilute 6 cc of glacial acetic acid to 100 cc with H_2O.
(c) *Ammoniacal silver nitrate soln.*—Mix 5 cc of 2% $AgNO_3$ with 5 cc of 10% NH_4OH.
(d) *Ammoniacal nickel acetate soln.*—Mix 1 volume of 5% $Ni(C_2H_3O_2)_2$ with 1 volume of 10% NH_4OH. Use the clear supernatant liquid.
(e) *Potassium thiocyanate soln.*—Dissolve 5 g of KCNS in 100 cc of H_2O.

PREPARATION OF SAMPLE

Separate the compound in pure form by the use of suitable solvents. Prepare a soln of a portion of the purified compound with the aid of acid, alkali, or H_2O as specified for the individual synthetic.

Controls.—For comparison, prepare a soln of the pure synthetic in the concentration specified for each.

IDENTIFICATION

To a drop of a soln of the compound or to about a mg of the powder on a glass slide, add a drop of the specified reagent. Without covering, examine for crystal formation under the microscope. Magnification of about 100 × is suitable. Note the characteristics of the crystals formed and compare with description and controls.

Characteristics of microchemical tests for synthetics

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Amytal	3% NH_4OH	1:50	Acetic acid	Long branching needles; some hexagonal plates in groups.
	3% NH_4OH	1:25	Acetic acid	Groups of rectangular plates.
Barbital				
	About 1 mg of powder		Ammoniacal silver nitrate	Stir to aid solution and crystallization. Very small twinned crystals and larger tufts.
	3% NH_4OH	1:50	Acetic acid	Dark burrs (stirring hastens crystallization).
Phenobarbital	About 1 mg of powder		Ammoniacal nickel acetate	Stir to aid solution and crystallization. Single rectangular crystals.
Ethylhydrocupreine	0.1 *N* HCl	1:100	Potassium thiocyanate	Long straight needles.

(3) The following method for the determination of hexylresorcinol was adopted as tentative:

HEXYLRESORCINOL

REAGENTS

(a) *Bromide-bromate soln.*—0.1 *N*. Dissolve 2.783 g of twice-crystallized $KBrO_3$ and 12.5 g of KBr in H_2O and dilute to 1 liter.

(b) *Sodium thiosulfate soln.*—0.1 *N*. Dissolve 25 g of $Na_2S_2O_3 \cdot 5H_2O$ and 0.2 g of $Na_2CO_3 \cdot H_2O$ in recently boiled and cooled H_2O and dilute to 1 liter. Standardize against the standard bromide-bromate soln.

(c) *Purified methanol.*—Add sufficient bromine vapor to commercial menthanol to give a bright yellow color and heat to boiling on the water bath for 5 min. Cool, and carefully decolorize by adding a 10% soln of $NaHSO_3$ dropwise until the methanol is just colorless.

(d) *Potassium iodide soln.*—Dissolve 20 g of KI in water and dilute to 100 cc.

(e) *Chloroform.*—U.S.P. XI.

STANDARDIZATION OF THIOSULFATE

Add 30 cc of the bromide-bromate soln to a 150 cc glass-stoppered volumetric flask. Add 10 cc of methanol. Wet the stopper. Add 5 cc of HCl, stopper the flask, immediately place under running tap H_2O, and swirl until the flask cools to room temp. Continue to shake the flask 5 min. after the HCl is added. Cautiously loosen the stopper and add 5 cc of the KI soln. Swirl gently to liberate the I, wash the stopper, and titrate with the thiosulfate. Add starch paste when the color of the soln is a pale yellow.

DETERMINATION

Transfer 0.07–0.09 g of sample to a 150 cc glass-stoppered volumetric flask. Add 10 cc of methanol and swirl gently to dissolve the sample. Add 30 cc of the bromide-

bromate soln. Moisten the stopper. Add 5 cc of HCl, stopper the flask, and immediately hold under running H_2O while swirling somewhat vigorously. When cooled to room temp. (about 1 minute), remove from the tap and continue to shake vigorously for 5 min. after the HCl is added. Cautiously loosen the stopper and add 5 cc of the KI soln. Swirl gently, wash the stopper with a little H_2O, add 1 cc of $CHCl_3$, and titrate with $Na_2S_2O_3$ while swirling the flask gently. Near the end point, stopper the flask and shake vigorously to get the free halogen out of the $CHCl_3$. When the color has been reduced to a pale yellow, add starch paste and continue the titration. The end point is reached when the starch-iodide color does not return during 30 seconds of vigorous shaking.

1 cc of 0.1 *N* bromide-bromate soln = 0.00488 g of hexylresorcinol.

THEOPHYLLINE
(Applicable to solutions and tablets)

(4) The following method for the assay of theophylline was adopted as tentative:

Weigh 0.2–0.3 g of theophylline (or a quantity of soln or powdered tablets containing an equivalent) into a separator. Add 5 cc of 0.5 *N* NaOH and shake the mixture gently until the alkaloid is dissolved. Add a strip of litmus paper and sufficient 0.5 *N* HCl from a buret to produce a distinct acid reaction. Then add 0.5 cc. more of the acid. Add 30 cc of chloroform-isopropyl alcohol mixture (3+1) and shake for 1 min. Allow to settle and draw off the lower layer into a second separator that contains 10 cc of H_2O acidified with HCl. Shake well, allow to settle, and filter the solvent into a weighed flask thru a pledget of cotton placed in the stem of a funnel. Repeat the extraction with 6 more portions of 20 cc each of the chloroform-isopropyl alcohol mixture, wash each portion thru the second separator, and pass the solvent thru the filter into the weighed flask. Insure complete extraction by a seventh shaking with 10 cc of the solvent and evaporation of the washed solvent in a separate container. Recover most of the solvent and evaporate the remainder on the steam bath while rotating the container in an inclined position. Add 2 cc of absolute ether to the residue and evaporate (cautiously to avoid spattering). Dry the residue at 80° to constant weight and weigh as anhydrous theophylline.

$$C_7H_8O_2N_4 \times 1.100 = C_7H_8O_2N_4 + H_2O.$$

(5) The following method for the assay of dinitrophenol (or its sodium compound) was adopted as tentative:

DINITROPHENOL (OR ITS SODIUM COMPOUND)

REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in 100 cc of H_2O.

(b) *Hydrochloric acid.*—35–37%.

(c) *Potassium iodide soln.*—Dissolve 20 g of KI in 100 cc of H_2O.

(d) *Bromide-bromate soln.*—0.1 *N*. Dissolve 2.7835 g of KBr in H_2O and dilute to 1 liter. If necessary, standardize against 0.1 *N* $Na_2S_2O_3$, *Methods of Analysis, A.O.A.C.*, 1935, 551, 26(c).

(e) *Sodium thiosulfate.*—0.1 *N*. Standardize as directed in *Methods of Analysis, A.O.A.C.*, 1935, p. 542, 3(b).

(f) *Starch soln.*—Mix 2 g of finely pulverized potato starch with cold H_2O to make a thin paste. Add 200 cc of boiling H_2O with constant stirring.

DETERMINATION

Weigh 0.18–0.20 g of 2–4 dinitrophenol (or sufficient of the preparation to contain that quantity) into a beaker of about 100 cc capacity, and dissolve the sub-

stance in 25 cc of H_2O, using sufficient 2% NaOH to insure soln. Transfer the soln to a 500 cc glass-stoppered flask, using H_2O for washing (do not use heat). Dilute the soln with H_2O to about 100 cc, add 25 cc of 0.1 *N* bromide-bromate soln and 10 cc of HCl. Immediately stopper the flask and swirl vigorously for 1–3 min. Remove the stopper quickly and add 5 cc of KI soln, taking care to avoid loss of bromine; immediately stopper the flask and shake thoroly for about 1 min. Remove the stopper and rinse down the neck of the flask with the H_2O. Titrate the soln with 0.1 *N* $Na_2S_2O_3$, using starch indicator near the end point.

1 cc of 0.1 *N* bromide-bromate soln = 0.0092 g of 2–4 dinitrophenol.

1 cc of 0.1 *N* bromide-bromate soln = 0.0103 g of sodium dinitrophenate.

1 cc of 0.1 *N* bromide-bromate soln = 0.0112 g of sodium dinitrophenate plus H_2O.

(6) The following method for the determination of cinchophen in the presence of sodium bicarbonate was adopted as tentative:

CINCHOPHEN IN PRESENCE OF SODIUM BICARBONATE

REAGENTS

(a) *Solvent.*—Mix 50 cc of alcohol and 50 cc of ether with 100 cc of $CHCl_3$.

(b) *Neutral alcohol.*—Neutralize 95% alcohol with 0.1 *N* NaOH to phenolphthalein.

DETERMINATION

Count and weigh a representative number of tablets, ascertain the average weight, and grind to a fine powder. Weigh a sample sufficient to yield 0.3–0.4 g of cinchophen, transfer to a separator, and add 10 cc of 4% NaOH soln to dissolve the cinchophen. Neutralize with 10% HCl and add 2 cc in excess Extract with five 25 cc portions of the solvent, collecting the extracts in a second separator. Wash with 25 cc of H_2O and filter the extracts into a beaker. Extract the wash water with 15 cc of solvent and filter into the same beaker. Test for complete extraction. Evaporate the solvent to dryness on the steam bath. Dissolve the residue in 60 cc of neutral alcohol. Titrate the soln with 0.1 *N* NaOH to a permanent pink color, using phenolphthalein as indicator.

1 cc of 0.1 *N* NaOH = 0.02491 g of cinchophen.

APPENDIX I. STANDARD SOLUTIONS

No additions, deletions, or other changes.

APPENDIX II. DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

See p. 44 for the definitions adopted as official this year.

No report was given by the Committee on Standard Scale for Immersion Refractometer.

No report was given by the Committee to Confer with American Public Health Association on Standard Methods of Milk Analysis.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

The Crop Protection Institute was organized September 28, 1920, or 16 years ago.

The purpose of the institute was to create a scientific agency that could make thorough and impartial tests of insecticides and fungicides for manufacturers and commercial firms.

New pests, conditions, and problems were constantly confronting farmers, and the existing methods and standard remedies were not always satisfactory or effective. It was desirable to devise means for more adequate control. The institute was set up to provide expert advice and technically trained scientists for forward looking manufacturers who desired to improve or develop and market materials meeting the needs of farmers for controlling crop pests.

The institute is under the direction of a Board of Governors composed of:

Three representatives of the American Association of Economic Entomologists
Three representatives of the American Phytopathology Society
Two representatives of the Association of Official Agricultural Chemists
One representative of the National Research Council.

ACCOMPLISHMENTS

An institute program initiated some of the research that has added to our knowledge of the properties and performance of sulfur. Institute projects have brought about notable advances in our knowledge of copper and copper compounds. Basic search of petroleum derivatives has been forwarded by institute projects, especially in territory east of the Rockies. Fundamental advances have been made in knowledge of compounds derived from the heavy metals. Through institute projects exploration of various groups of organic chemicals has been initiated.

The work of the institute has brought about among manufacturers a new appreciation of the value of research from a biological point of view.

NEW DEVELOPMENTS

Specific new materials developed in whole or in part through institute projects constitute a considerable list. Thus, much of the development work with the household insecticide Flit was done through the institute. The dormant oil spray Dendrol and the summer oil spray Verdol are the results of institute projects. Other developments include the following: The new fumigating gas Proxate, the colloidal copper compound Coposil, a further colloidal copper compound now in its final stages of development, an impregnated tape for use in wrapping nursery grafts, flotation sulfur, improved formulas for cattle sprays, the use of dilute sulfuric acid in large-scale weed control, the oxydized oil Penetrol, a series of new materials known as Dowsprays, the utilization of basic zinc arsenate in

codling moth control, and the use of copper sulfate in fertilizers. Institute work resulted in noteworthy contributions to our knowledge of crown gall. Institute projects have furnished important information concerning the performance of oil sprays on plants, the inflammability of various mixtures of gases, the performance of chlorinated naphthalene, and the methods of determining and rating toxicants in spray materials.

COOPERATION

Through the institute friendly contacts between the technical men in industry and those in the biological sciences have been furthered. The result of this has been a greater understanding on the part of industrial men as to the nature and significance of the work done by the experts in experiment stations, and equally better comprehension on the part of the experiment station men as to the problems and achievements of the technical men in industries.

A list of projects carried on by the institute was given in the reports submitted in 1934 and 1935. A few changes have occurred in this list owing to the completion of some and the adding of new ones, but these lists serve to give a good idea of the character, extent, and distribution of the institute's activities.

Approved.

H. J. PATTERSON
W. H. MACINTIRE

REPORT OF SECRETARY-TREASURER

During the year there were a number of changes in the referees and associate referees. These appointments were made by the secretary in the usual manner, and the names of the appointees and subjects were published later in *The Journal*. There were four expired terms in the subcommittees of the Committee on Recommendations of Referees and one on the Editorial Committee of *The Journal*. The president will announce these new or reappointed members later.

We have on hand 350 copies of the 1930 edition of *Methods of Analysis*. As you know, last year the Association authorized the sale of the remaining copies at $2.50 per volume. There has been a rather active demand for these books for textbooks in some schools and colleges, and I anticipate that we shall dispose of these additional copies. As has been reported to you, 5000 copies of the 1935 edition of *Methods of Analysis*, revised under the immediate direction of E. M. Bailey, have been printed, and 3000 copies have been bound and are ready for distribution. By vote of the Executive Committee, after a canvass had been made of the financial condition of the Association, it was decided to sell this book to members for $4 instead of $5. This will reduce our income about $1500 on an issue, but I think we can afford it, as you will see when I read the treasurer's report. This is not a money-making organization, and it has

not been the policy of the Association to accumulate any large surplus. It is only necessary to have a reserve to meet the publication needs for *The Journal* and *Methods of Analysis*. It will be of interest to all our members to know that we have a cash balance sufficient to meet the payment for the fourth edition of *Methods of Analysis* upon delivery.

There has been some discussion by the Executive Committee in regard to using some of our surplus in the publication of monographs covering certain activities in which the Association is interested. This matter was also mentioned in the report of the Editorial Board. Final action on this project has been deferred until more definite arrangements have been made.

By direction of the Executive Committee the treasurer was authorized three years ago to have the accounts of the Association audited by a public accountant. This was a wise provision because the activities and business affairs of the Association have grown until we are now quite a large organization. Some three or four years ago it was also deemed wise to incorporate the Association, which was done. The elected officers of the Association were made directors of the corporation.

The Executive Committee approved the expenditures shown in the following report received from John W. Bisselle, 1331 G St. N. W., Washington, D. C., public accountant:

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR THE YEAR ENDED SEPTEMBER 30, 1936

Balance, October 1, 1935:			
Lincoln National Bank		$2,023.89	
Montgomery Building Association		1,552.65	$ 3,576.54
RECEIPTS			
Sales:			
Methods	$2,214.51		
Journals	4,097.60		
Reprints	256.62		
Wiley's Principles	15.00	$6,583.73	
Less: Discounts Allowed		631.95	
Net Sales			5,951.78
Other Income:			
Advertisements		$ 280.00	
Interest Income:			
U. S. Treasury Bonds	$ 194.99		
Federal Land Bank Bonds	140.00		
Home Owners Loan Corporation Bonds	55.00		
Montgomery Building Association	99.85	489.84	
Total Other Income			769.84
Miscellaneous Receipts:			
Books ordered through Association			504.11
			$10,802.27

DISBURSEMENTS

Expenses:			
Salaries		$1,105.00	
Postage		275.00	
Meeting and Association expense		208.05	
Traveling expense		105.00	
Stationery and supplies		152.43	
Auditing		135.00	
Premiums, employees' bonds		12 50	
Safe deposit rental		3 30	
Exchange		4.33	
Printing:			
Reprints	$ 417.14		
Journals	3,434 57	3,851.71	
Binding		6.00	
Sales refunds		6 00	
Total Expenses			$ 5,864.32
Miscellaneous disbursements:			
Books ordered through Association		$ 495 65	
Returned checks		9 00	
Warrant, City of Milwaukee		4 00	
Total Miscellaneous Disbursements			508 65

BALANCE, SEPTEMBER 30, 1936

Lincoln National Bank	$4,376.80	
Montgomery Building Association	52 50	4,429.30
		$10,802.27

BALANCE SHEET AS AT SEPTEMBER 30, 1936

ASSETS

Current Assets:			
Cash in Banks:			
Lincoln National Bank	$4,376.80		
Montgomery Building Association	52.50	$4,429.30	
Unredeemed warrants not due		4.00	
Accounts receivable	$2,585 97		
Less: Reserve for Doubtful Accounts	120 80	2,465 17	
Inventories		4,817.17	
Total current assets			$11,715.64
Investments:			
Home Owners Loan Corporation Bonds		$1,000.00	
U. S. Treasury Bonds		5,000 00	
Federal Land Bank Bonds		4,000.00	
Total investments			10,000.000
Cash in closed banks:			
Federal-American Bank and Trust Company		$ 36 93	
Commercial National Bank		120 42	157.35
Total assets			$21,872.99

LIABILITIES AND SURPLUS

Current Liabilities:

Accounts Payable	$ 6.55
Surplus	21,866.44
Total liabilities and surplus	$21,872.99

Approved.

W. W. SKINNER

H. H. Hanson: Our secretary spoke of some appointments made by the Executive Committee. I will read them at this time. R. B. Deemer was reappointed on the Editorial Board of *The Journal.* On the Committee on Recommendations of Referees, H. A. Lepper was reappointed chairman. On each of the four subcommittees, A, B, C, and D, the term of one member had expired. On Committee A, H. A. Halvorson was appointed; on Committee B, A. E. Paul was reappointed; on Committee C, W. B. White was reappointed; and on Committee D, J. W. Sale was reappointed.

REPORT OF THE AUDITING COMMITTEE

The public accountant's audit of the books of the Association of Official Agricultural Chemists, Inc., as of September 30, 1936, was examined by the Committee and found to be correct. Verification was also made of the bonds on deposit.

H. A. HALVORSON
JOHN B. SMITH

Approved.

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS

During the years 1934 and 1935 no meetings were held. The first since that of April, 1933, occurred during the week of September 28, 1936, this being the 44th. A rather wide variety of topics was given consideration.

The existent definition for orange juice was modified by deletion of the words "and/or of the volatile oil." This definition now reads:

Orange juice. The unfermented juice obtained from sound, ripe, sweet oranges. It may contain a portion of the pulp.

At the 1933 meeting tentative definitions were formulated for commercial egg products. As now given final adoption, this schedule is as follows:

Liquid Egg, Mixed Egg. The product obtained by separating the edible portion of eggs from the shells. It is an intimate mixture of the whites and yolks in their natural proportions.

Frozen Egg. The solidified product obtained by quickly and completely freezing liquid eggs.

Dried Egg. The product obtained by evaporating the water from liquid eggs. It contains not more than 7% of moisture.

Egg Yolk. The product obtained by removing the whites from the yolks in the commercial process of egg-breaking. It contains not more than 12% by weight of adhering white.

Frozen Egg Yolk. The solidified product obtained by quickly and completely freezing egg yolks.

Dried Egg Yolk. The product obtained by evaporating the water from egg yolks. It contains not more than 5% of moisture.

New and revised definitions proposed are:

Orangeade. The beverage consisting of orange juice, sugar and water It contains not less than 25 per cent of orange juice. The acidity may be increased by the addition of lemon juice.

Lime Rickey. The beverage consisting of lime juice, sugar and carbonated water. It contains not less than 7 per cent of lime juice.

Molasses. The product which remains after separating sugar from the clarified and concentrated juice of the sugar-cane. It may be "light" or "dark." It contains not more than 25 per cent of moisture and not less than 55 per cent of total sugars (sucrose plus reducing sugars). Reducing sugars are calculated as invert sugar.

Light Molasses is molasses that contains not less than 62 per cent of total sugars.

Dark Molasses is molasses that contains not less than 55 per cent of total sugars.

Cream Cheese. The soft, uncured cheese made from curd obtained by the action of either lactic fermentation or rennet, or both, on milk enriched with cream. The curd, heated or unheated, salted or unsalted, is drained by gravity and light pressure. The finished product contains not more than 52 per cent of water and, in the water-free substance, not less than 65 per cent of milk fat.

Requests were received, largely from trade sources, for definitions, or revision of present ones, for a wide variety of products, among them Hungarian paprika, fresh vegetables, sweet pickled fruits, chocolate milk, Worcestershire sauce, common salt, toffee, honey, imitation flavoring extracts, Swiss cheese, process cheese, and dried milk. These were considered and indefinite postponement agreed upon. Definitions for some of these items were deemed inexpedient, and for others information was insufficient to warrant action.

Anticipating a need for revision of "Service and Regulatory Announcements, Food and Drug No. 2, Fourth Revision" (food standards), attention was given to editorial changes in a number of schedules, none, however, changing the meaning of present language. As a matter of policy it was decided to abandon the present plan of definition whereby the article defined becomes the subject of a sentence. In the interest of greater clarity it was agreed to adopt the dictionary style, provided no change in present meaning is involved.

C. D. Howard
E. M. Bailey
Guy G. Frary

Approved.

REPORT OF COMMITTEE ON NECROLOGY

Since our previous meeting two losses have occurred among those who in past years have participated in the work of our Association. These deceased colleagues are Doctors C. B. Morison and C. E. Mangels, whose deaths last April, only six days apart, came as a great shock to their many friends and coworkers.

C. Brewster Morison was born in Norwich, Conn. on Sept. 27, 1882, of old New England ancestry. He graduated from Kingston College, R. I., and after doing post-graduate work at Brown and Yale Universities occupied a position on the chemical staff of the Connecticut Agricultural Experiment Station in New Haven for the next 11 years. During the World War he served as first lieutenant in the sanitary division of the medical corps. In 1919 he became associated with the American Institute of Baking, first as Assistant Director and then since 1923 as Dean. He died on April 17, 1936, of heart disease after an illness of four months. Dr. Morison was an active worker in the field of cereal chemistry and during the past 15 years had been a regular attendant at the meetings of the A.O.A.C. He was a man of scholarly attainments and a collector of rare books relating to the science of nutrition. The members of this Association will long recall the kindly genial personality of Brewster Morison.

Charles Edwin Mangels also achieved distinction in the field of cereal chemistry. He was born at Hannibal, Missouri, on April 24, 1892. He obtained his B.S. degree in 1914 at the University of Missouri, at which he then worked two years as assistant agricultural chemist. In 1916–17 he worked at the Ohio Experiment Station and then taught agricultural chemistry for a brief period at the University of Missouri. At the outbreak of the World War he accepted a position as second lieutenant in the Sanitary Corps. From 1919 to 1921, Dr. Mangels had charge of the dehydration work of the U. S. Bureau of Chemistry. In 1921 he accepted the position of cereal chemist at the North Dakota Agricultural Experiment Station and continued at this post until his death on April 23, 1936. He was awarded the degree of Ph.D. by the University of Minnesota in 1932. Dr. Mangels was only an occasional attendant at the meetings of the A.O.A.C. He was co-author with P. F. Trowbridge at the 1915 meeting of a paper on "The Determination of Crude Fiber," *This Journal*, **2**, 132 (1916), and contributed reports as Referee on Cereal Foods at the 1923 meeting, *Ibid.*, **8**, 148 (1924), and on Ash in Cereal Products at the 1924 meeting, *Ibid.*, 671. He was an active worker in the American Association of Cereal Chemists, of which he was President in 1928–9.

Approved. C. A. BROWNE

REPORT OF NOMINATING COMMITTEE

The Committee on Nominations submits the following report.

President: C. C. McDonnell, Washington, D. C.

Vice-President: H. R. Kraybill, Lafayette, Ind.

Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

W. S. Frisbie, Washington, D. C.

C. L. Hare, Auburn, Ala.

L. B. Broughton, College Park, Md.

Post-Officio:

H. H. Hanson, Dover, Del.

W. H. MacIntire
L. S. Walker
A. E. Paul

A unanimous vote was cast for the officers nominated.

The other appointments were announced by the president (see p. 88).

REPORT OF COMMITTEE ON RESOLUTIONS

Whereas, our Association is about to conclude its fifty-second annual meeting; and

Whereas, our formal and informal discussions of an unusually comprehensive program make it possible for us to return to our official duties better equipped to perform them; be it

Resolved: That we express to our president, Mr. H. H. Hanson, our appreciation of his very able presidential address, and of the dignified and efficient manner in which he has presided over our deliberations.

Resolved: That we express our pleasure in having with us again our former members, Dr. and Mrs. A. L. Winton; and our sincere thanks to Dr. Winton for his scholarly Wiley Memorial Address on "Structure as an Approach to Food Chemistry."

Resolved: That we express our appreciation to all members of our Executive Committee, and especially to Dr. Skinner and Miss Lapp for the thought and attention given by them to insure the interest and success of our meeting.

Resolved: That we extend our thanks to Mr. W. J. D. Walker of the Corning Glass Works, for his very interesting talk on, and demonstration of, the art of glass blowing.

Resolved: That, through our Secretary, we extend our thanks to the management of the Raleigh Hotel for all the courtesies shown our members and guests.

E. M. BAILEY
H. R. KRAYBILL
H. B. MACDONALD

Approved.

CONTRIBUTED PAPERS

DEVELOPMENT OF METHODS FOR THE ESTIMATION OF MOLD IN CREAM OR BUTTER

By J. D. Wildman (Microanalytical Division, U. S. Food and Drug Administration, Washington, D. C.)

The need for simple methods of estimating the amount of mold mycelium in cream and butter led to the development of the two procedures described in this paper. The laboratory work was begun in November 1934. At different times since then, the field work was done at creameries located in the eastern and central states. Part of the work was carried on at the Purdue University creamery.

MICROSCOPIC METHOD FOR ESTIMATING MOLD IN BUTTER AND CREAM

Butter.—The development of the microscopic method for estimating the amount of mold in butter proceeded by various stages. In the first experiments the butter was heated and the fat removed by decantation. To the remaining curd and serum was added sufficient 50 per cent glycerin solution to bring the volume of the preparation up to that of the original butter used, after which a drop of the thoroughly mixed sample was mounted on a Howard counting chamber and an estimation made of the relative number of mold filaments by the Howard method. The necessity of prolonged heating of the butter to remove the fat was next eliminated by the use of gasoline. It was found at this stage that certain butter samples showed numerous mold filaments in all fields. To reveal any significant differences in mold content between samples of this character, it was then necessary to dilute the serum-glycerin mixture with seven parts of glycerin solution. Further study, however, showed that it was not necessary to remove the fat since by this dilution the fat globules were so separated that the mold filaments could be readily seen. Finally, a gum solution was substituted for the glycerin. As used at the present time, the complete method is as follows:

EQUIPMENT AND REAGENTS

(a) *Measuring spoon.*—¼ teaspoon.

(b) *Pipet.*—10 cc.

(c) *Gum solution.*—Make up 1 liter of a 0.75% solution of carob bean gum with 2% of added formaldehyde as a preservative. Use the clear supernatant solution, free from cells, left when the cellular elements in the gum gradually settle out. (A similar solution made with gum tragacanth may also be used for this purpose.)

PROCEDURE

Weigh out 1 gram of butter by means of the ¼ teaspoon measure. Measure out 7 ml. of hot gum solution and, with the spoon bottom side up over a 50 ml. beaker,

pour 2 or 3 ml. of the hot solution over the spoon. (This quantity is usually sufficient to loosen the butter and cause it to slide into the beaker.) Use the remainder of the 7 ml. of solution to rinse the remaining fat from the spoon.

Stir until the solution is well mixed and fat globules are 0.1–0.2 mm. in diameter. (The stirring necessary to obtain a uniform sample must be determined by experience.)

Mount a portion of the mixture on the mold-counting slide and estimate the mold by the Howard method.[1] Report no field as positive unless the combined length of the two longest filaments exceeds ⅙ of the diameter of the field.

Cream.—The method that has been used to make mold counts on cream is indirect, *i.e.*, the cream is first churned and then the mold count is determined on the butter.

Many tests on butter and cream were made by means of the microscopic method previously described. In following the results given here, the reader should bear in mind that this method represents a somewhat different approach to the problem of mold estimation in butter, inasmuch as other microscopic methods have been directed primarily to the estimation of mold spores and segments and a detection of secondary contamination, and not to the estimation of the mold growth that had occurred in the cream.

MOLD COUNTS ON BUTTER FROM SAME CHURN

Since only a relatively small quantity of butter is taken for an individual test in the method presented here, the question naturally arises as to whether such a sample is representative of the total amount of butter in the churn. To test this point experimentally, a number of counts were made, the results of which are given in Table 1. Samples of approximately

TABLE 1.—*Mold counts on butter samples taken from the same churn*

CREAMERY NO.	SAMPLE NO.	LOCATION	NO. FIELDS COUNTED	MOLD COUNTS
1		Central States	50	2, 0, 2, 4, 6, 4, 6, 0, 4, 2, 2, 2
2		Central States	50	12, 14, 10, 8, 12, 10, 14, 10, 12, 14, 14, 12
3		Southern States	50	62, 60, 57, 58
4	1	Eastern States	100	40, 43, 39, 43, 41
4	2	Eastern States	100	20, 19, 20, 17, 17
4	3	Eastern States	100	34, 34, 34, 30, 37
4	4	Eastern States	100	18, 17, 22, 20, 19
4	5	Eastern States	100	30, 32, 33, 31, 33
5	1	Central States	100	86, 86, 85, 80, 79
5	2	Central States	100	49, 43, 53, 51, 44
5	3	Central States	100	20, 20, 23, 19, 19
6		Central States	100	19, 19, 19, 21, 22
7		Central States	100	6, 5, 5, 2, 6

[1] *Methods of Analysis, A.O.A C*, 1935, 500.

10 grams each, varying in number from four to twelve, were taken at random from various parts of the mass of finished butter. It is apparent from the table that the counts on any one churn are consistently close together and that the mold is in general very evenly distributed throughout the butter.

MOLD COUNTS ON COMMERCIAL BUTTER

Some idea of the amount of mold in commercial butters may be obtained from Table 2 and Chart 1. The counts were obtained on 65 commercial samples, taken at random in order to represent as nearly as possible a cross section of the butter market, and on 53 samples that were taken from lots that had been seized for mold or filth. It will be seen that the bulk of the seizure samples counted from 50 to 90 per cent, while the bulk of the random samples varied from 0 to 30 per cent.

TABLE 2.– *Mold counts on commercial samples*

LIMIT OF MOLD COUNT	RANDOM SAMPLES*		SEIZURE SAMPLES	
per cent	*No.*	*per cent*	*No.*	*per cent*
0– 20	53	81.5	0	0 0
20– 40	5	7 7	2	3.7
40– 60	5	7.7	15	28.3
60– 80	1	1 5	20	30.7
80–100	1	1 5	16	30 2
	65		53	

* The writer is indebted to F A Hodges for the counts made on the random series and also for assistance in some of the field work.

MOLD CONTENT OF CREAM DELIVERIES

To obtain light on the question of the frequency and extent of the occurrence of mold in cream from individual producers, samples were collected at Creamery No. 4 on December 14, 1934; May 16, 1935; July 6, 1935; and November 6, 1935, and at Creamery No. 8 (Central States) during the week beginning August 1, 1935. In all, about 144 such samples were taken. These were churned by hand, and mold counts were made on the butter. Some doubt may arise as to whether the churning method used gave results comparable to what would have been secured had commercial churning methods been employed, but since all samples were treated essentially alike it is believed the results reflect the relative conditions fairly well. The results obtained are given in Table 3 and Chart 2. When all the samples are considered collectively, irrespective of the season, it will be seen that the majority of the samples have relatively low mold counts.

TABLE 3.—*Mold counts on cream deliveries*

DATE OF SAMPLING	SOURCE	% OF SAMPLES WITH MOLD COUNTS FALLING BETWEEN				
		0–20	20–40	40–60	60–80	80–100
December 14, 1934	Creamery No. 4	95	5	0	0	0
May 16, 1935	Creamery No. 4	90	5	5	0	0
July 6, 1935	Creamery No. 4	77.5	7.5	7.5	2.5	5.0
August 6, 1935	Creamery No. 8	54.5	18.2	6.8	6.8	13.7
November 6, 1935	Creamery No. 4	57.9	31.6	0	0	10.5
Total of all dates		72.3	13.2	4.8	2.8	6.9

The effect of season is shown in Chart 3. The figures indicate that in the sections studied there is a tendency to produce mold-containing butter mainly during the summer and fall months.

EFFECT ON CHURNING ON MOLD

To determine the effect of churning on the distribution of mold in the butter and buttermilk, the following experiments were made.

A sample of moldy cream was churned. It yielded butter and buttermilk in the ratio of 2 to 3. The mold count on the butter was 76 per cent, while on the buttermilk it was only 4 per cent. In order to approximate the count on the original cream, a subsample was then prepared by mixing two parts of the melted butter with three parts of the buttermilk. The mold count on this mixture was 60 per cent. These results clearly show that in this case churning, instead of reducing the amount of mold, actually concentrated it in the butter. The tests recorded below on other samples indicate further that the mold mycelium is retained in the butter to a great extent.

Sample No	*Buttermilk per cent*	*Butter per cent*
1	4	40
2	4	92
3	24	68

MACROSCOPIC TEST FOR MOLD IN CREAM

In addition to the work on the microscopic method for the estimation of mold in cream and butter, considerable time was spent in developing a method for the macroscopic detection of mold in cream to provide the creamery operator, if possible, with a rapid, simple means of detecting moldy cream in those cases where the mold had been stirred into the cream.

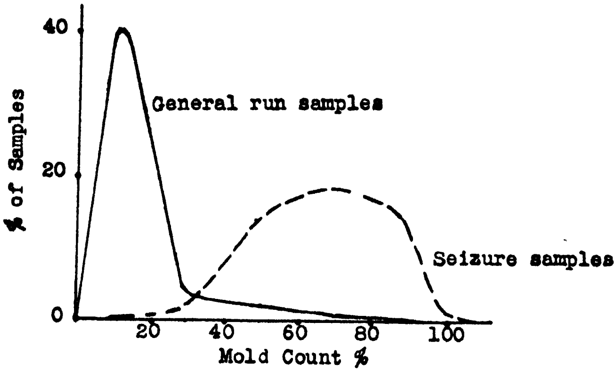

CHART 1.—MOLD COUNTS ON CREAMERY BUTTER

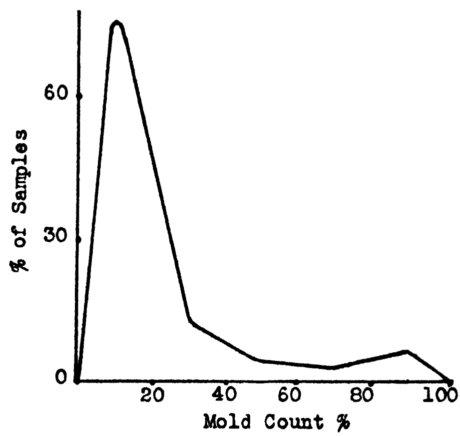

CHART 2.—MOLD COUNTS ON CREAM DELIVERIES

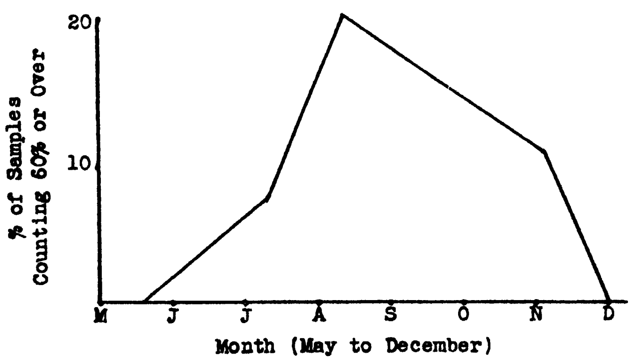

CHART 3.—INCREASE IN NUMBER OF HIGH COUNTING SAMPLES DURING SUMMER AND FALL

The test used for the work described in this paper follows:

Method "A"

EQUIPMENT AND REAGENTS

(a) *Teaspoon.*—5 ml.

(b) *Methylene blue-borax solution.*—Dissolve 30 grams of sodium borate and 10 methylene blue tablets (as used for reductase test) in 1 liter of water.

(c) *Test tube.*—5–6".

(d) *Shallow tin pie pan.*—Approximately 7" in diameter.

(e) *Scalpel.*

PROCEDURE

Measure out 5 ml. of cream, preferably warm as for Babcock test sampling, in the teaspoon and place in the test tube. Add 15 cc. of hot (90°C.) methylene blue-borax solution, using a portion to rinse all the cream from the teaspoon into the tube. Shake the mixture well and pour into the pie pan. Tip the pan back and forth until all mold present has agglutinated into blue stained masses. (In samples containing much mold, the flocculation occurs immediately. In most cases of non-moldy sour cream, no blue flecks will form.) For purposes of comparison, collect the mold into a disk with a scalpel, and measure and designate the diameter roughly by the following scheme:

Diameter of Clot *mm.*	*Approximate Area* *sq. mm.*
9.5	70
5.5	24
3.5	9
2.5	4
1.0	1

At the present time the following simpler method of examination is used:

Method "B"

EQUIPMENT AND REAGENTS

(a) *Beaker.*—50 cc.

(b) *Methylene blue-borax solution.*—Prepare as directed in Method "A."

(c) *Perforated funnel.*—Made from perforated brass, No. 26 gage, having approximately 625 holes per sq.in., each hole being 0.02" in diameter. A circular disk 1.5" in diameter is cut, and from it is removed a 45° sector. The edges of the remaining disk are bent together until they meet, and are then soldered. To this funnel is soldered a 0.5" collar.

(d) *Scalpel or dissecting needle.*

PROCEDURE

Weigh out 5 grams of cream into the beaker and add 15 cc. of the hot methylene blue-borax solution. After agitating the beaker for 3 minutes, pour the mixture through the special perforated funnel. Add more hot methylene blue-borax solution to wash any mold clots into the apex of the funnel. By means of a dissecting needle, level off the top of the mold until it forms an inverted cone and then measure the diameter of the base formed thereby (mm.).

RELATION OF MBB TEST ON CREAM TO MOLD COUNT ON BUTTER

For convenience, the test described for the examination of cream for mold has been termed the MBB test (methylene blue-borax). In order to

determine what relation, if any, exists between the MBB test on cream and the mold count on the butter, mold counts were made on 44 individual samples of butter churned from cream upon which MBB tests had previously been made. This work was done in Creamery No. 8. The results obtained are shown in Table 4.

These methods depend upon the tendency of mold to flocculate in the presence of an alkaline solution. When present in sufficient numbers, yeast and certain bacteria are also agglutinated by the treatment, and therefore it is not surprising that an occasional cream sample gives a high test by the MBB method but fails to give a high mold count by the microscopic method when churned into butter. Thus, it is possible that high MBB tests, whether showing mold or not, may indicate objectionable cream because of the other microorganisms present.

TABLE 4.—*Relation of MBB test on cream to mold count on butter*
(Data obtained at Creamery No. 8, 1935)

DIAMETER OF MOLD CLOT	INDIVIDUAL MOLD COUNTS	AVERAGE MOLD COUNT
mm.		
—	0, 0, 2, 0, 0, 0, 2, 18, 16, 18, 2, 8, 0, 28, 8, 6.	6.7
1.0	0, 38, 18, 44, 12, 20, 12, 2, 10, 6.	16.2
2.5	36, 26, 24.	28.6
3.5	30, 88, 20, 56, 68, 10.	45.3
5.5	60, 14, 52, 90, 100.	63.2
9.5	80, 60, 100, 98.	84.5

It is seen (Table 4) that those samples showing low MBB tests also show low mold counts and that, in general, with higher MBB tests higher mold counts were obtained.

RELATION OF MBB TEST TO QUALITY OF CREAM

In order to obtain some information regarding the relation of the MBB test to the quality of cream, the writer ran the methylene blue-borax test on 235 cream samples taken at random at Creamery No. 8 from the samples previously graded by the creamery attendant employed for that purpose. After each day's MBB tests had been made, the cream-grading sheets were consulted, and the grade of the cream and remarks by the grader concerning the flavor were recorded.

Off flavor conditions noted by the grader were the following: Oily, cheesy, musty, bitter, soapy, fruity, weedy, yeasty. In Table 5 are summarized the results of the MBB tests. All samples showing any of the above flavors are lumped together under the heading of "Off Taste." The MBB tests are combined into three groups as follow:

Negative.—No mold debris.

Medium.—Mold clots with diameters of 1, 2.5, and 3.5 mm. areas. This averages nearly 5 sq. mm. of mold debris per 5 cc. of cream.

Large.—Mold clots with diameters of 5.5 and 9.5 sq.mm. Approximately 50 sq.mm. of mold debris per 5 cc. of cream.

TABLE 5.—*Relation of off tastes in cream to MBB test*

TASTE	PER CENT OF SAMPLES SHOWING MBB TEST OF—		
	NEGATIVE	MEDIUM	LARGE
Normal Sour	32.0	11.2	0.4
Off Taste	23.0	22.2	11.2

These figures show that "off tastes" tend to be associated with the occurrence of mold. The fact that 23 per cent of the samples was off taste but gave negative results by the MBB test merely emphasizes the generally recognized fact that other factors than mold may cause off flavors in cream. It is evident, therefore, that a low MBB test does not necessarily indicate good cream.

Of the 235 samples examined, 27 were found by the MBB test to have large amounts of mold debris. Of these 27, only one was passed by the grader as being normal sour, the rest being characterized by off flavor and of such character that in the grader's opinion they were not suitable for the manufacture of No. 1 butter. In other words, in this series, at least, large amounts of mold debris by the MBB test were accompanied in 97.3 per cent of the cases by abnormal flavors. Whether the mold was alone responsible for the occurrence of these flavors obviously cannot be determined from this type of experiment.

SUMMARY

A microscopic method for the estimation of the amount of vegetative mold in butter and a simple macroscopic method for the detection and estimation of the amount of mold in cream are described. The principal points brought out in the development of these methods are the following:

(1) Mold was generally so well distributed throughout the churn that results on one sample by the microscopic method were considered representative of the whole churning.

(2) The mold counts on certain samples of butter seized because of mold and filth were distinctly higher than those obtained on a series of butters sampled at random.

(3) At creameries where tests were made, it was found that the majority of the cream samples taken from individual farmer's lots had a relatively low mold content as shown by low mold counts. During the hot summer months and the fall, however, the proportion of high counting samples increased.

(4) The vegetative mold in cream was retained to a large extent in the butter on churning.

(5) By the MBB (methylene blue-borax) test, it was possible to detect cream samples containing excessive amounts of mold.

(6) It was found that 97 per cent of the samples showing high MBB tests had abnormal flavors.

USE OF SILICA DISHES IN OFFICIAL METHOD FOR DETERMINATION OF POTASH IN FERTILIZERS

By H. R. Allen and Lelah Gault (Kentucky Agricultural Experiment Station,* Lexington, Ky.)

Haigh,[1] using the method for the determination of potash official in 1928, obtained lower results with ignition in silica dishes than he did with platinum dishes. The action of the metaphosphates or free phosphoric acid formed may have rendered some of the potash insoluble. In the present official method,[2] sodium hydroxide, added to the solution before ignition, prevents the formation of free phosphoric acid. Kraybill and Thornton[3] found practically no loss in weight of silica dishes when sodium hydroxide was added, but there was considerable loss when it was omitted.

Samples of complete fertilizers, obtained by inspectors for the Department of Fertilizer Control, furnished the potash used in this study. Nearly all the samples contained organic material. Portions of 25 ml. each from the same solution of each sample were delivered into platinum and silica dishes. The ignition was conducted at the lowest practical temperature. The dishes were placed on a hot plate and heated slowly until frothing began, when they were transferred to burners equipped with the Purdue burner top,[4] and the heat was gradually increased until all organic material was removed from the sides of the dishes. Ignition was continued to whiteness over Tirrill burners, and the hot cone of the flame was kept about 2 inches below the bottom of the dish. No difficulty was experienced in obtaining a perfectly white residue in all cases. The full heat of the burner was not required. Results are given in Table 1.

Analysis of Materials of Known Potash Content.—As a check on the method, the potash content of mixtures of potash salts and a superphosphate was determined. To avoid inequalities due to mixing, 0.6250 gram of the potash salt and 1.4 grams of superphosphate were transferred directly to the 250 ml. flask for digestion. A pure potassium chloride and a pu1 potassium sulfate, dried at 120°C., were used. Their potash content was determined by the usual method for such salts. Silica dishes were used. Results are given in Table 2. Each figure is the average of two or more analyses.

Effect of Re-washing Filter Pads.—A rather large insoluble residue is left in some cases after the K_2PtCl_6 has been dissolved. This may be due, in part, to colloidal material not retained by the filter paper, which was Whatman Folded No. 18. After standing several days, some of the potash

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director Presented at the Annual Meeting of the Association of Official Agricultural Chemists, December, 1936.

[1] *This Journal*, 9, 219 (1928)
[2] *Ibid.*, 19, 308 (1936)
[3] *Ibid.*, 18, 272 (1935).
[4] *Ind. Eng. Chem. Anal. Ed*, 7, 119 (1935).

TABLE 1.—*Comparison of platinum and silica dishes in potash determination*

LABORATORY NUMBER	K_2PtCl_6			INSOLUBLE RESIDUE*	
	PLATINUM DISH	SILICA DISH	DIFFERENCE	PLATINUM DISH	SILICA DISH
	gram	*gram*	*mg.*	*mg.*	*mg.*
7261	.0415	.0422	+0.7	3.2	7.3
9168	.0470	.0470	0.0		
7332	.0510	.0516	+0.6	3.1	4.2
7340	.0528	.0532	+0.4		
9096	.0548	.0560	+1.2		
9078	.0618	.0629	+1.1		
7260	.0667	.0665	−0.2	2.7	2.5
9084	.0711	.0715	+0.4	4.5	8.8
9083	.0721	.0722	+0.1		
9011	.0731	.0748	+1.7		
7329	.0738	.0748	+1.0	2.7	2.0
7254	.0745	.0746	+0.1		
9335	.0794	.0812	+1.8	4.0	3.0
9097	.0801	.0805	+0.4		
9388	.0878	.0872	−0.6	0 3	0.6
9110	.0969	.0970	+0.1	6.6	3.4
9274	.1105	.1112	+0.7	3.6	5.0
7335	.1157	.1171	+1.4		
9073	.1194	.1216	+2.2		
9405	.1367	.1366	−0.1	4.6	3.0

* Increase in weight of crucible after K_2PtCl_6 was washed out.

solutions showed a sediment in the bottom of the container. In the latter part of this investigation a double folded filter paper was used and the solutions contained no sediment after standing. In a series of determinations showing large insoluble residues, the filter pads were re-washed twice with acid alcohol, five times with ammonium chloride (35 cc.), four times with 80 per cent alcohol, dried, and weighed. Results are given in Table 3.

In a similar series of four determinations, the filter pads were washed five times with acid alcohol, four times with 80 per cent alcohol, dried,

TABLE 2.—*Results of analysis of materials of known potash content*

MATERIALS	K_2PtCl_6		INSOLUBLE RESIDUE
	FOUND	THEORY	
	gram	*gram*	*mg.*
KCl	0.2031	0.2034	0.2
K_2SO_4	0.1740	0.1743	0.1
Superphosphate	0.0014		1.0
KCl and Superphosphate	0.2044	0.2048	0.3
K_2SO_4 and Superphosphate	0.1752	0.1757	0.1

TABLE 3.—*Effect of re-washing filter pads*

NUMBER	GAIN IN CRUCIBLE WEIGHT AFTER K_2PtCl_6 WAS WASHED OUT	LOSS IN CRUCIBLE WEIGHT AFTER SECOND WASHING
	mg.	*mg.*
1	8.0	0.6
2	9.0	0.7
3	7.7	0.3
4	7.7	0.4
5	6.0	0.2
6	4.3	0.3
7	5.5	0.2
8	2.5	0.2
9	4.4	0.5
10	7.8	0.2

and weighed. Average loss in weight after the second washing was 0.2 mg. In another series, the pads were washed six times with boiling water, twice with 80 per cent alcohol, dried, and weighed. Average loss in weight of the crucibles after the second washing for twelve samples was 0.16 mg.

A series of analyses was made to compare ignition with (1) Purdue burner top and Tirrill burner; (2) Purdue burner top and Meker type (Fisher) burner; and (3) electric muffle. Dishes ignited in the muffle were first placed over the Tirrill burner and heated carefully until practically all fumes were removed. In all cases, dishes were placed about 2 inches above the hot part of the flame. All samples except No. 9366 contained organic material. Results are given in Table 4.

TABLE 4.—*Results of various methods of ignition*

NUMBER	METHOD OF IGNITION					
	PURDUE BURNER TOP AND MEKER BURNER		PURDUE BURNER TOP AND TIRRILL BURNER		TIRRILL BURNER AND ELECTRIC MUFFLE	
	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE
	gram	*mg.*	*gram*	*mg.*	*gram*	*mg.*
9277	0.0516	0.4	0.0512	0.3	0 0513	0 7
9270	0 0544	3.2	0.0535	2.7	0.0541	2.3
9333	0 0720	1.4	0.0727	0 5	0.0712	4.9
9324	0.0775	0.6	0.0766	0.0	0.0767	3.5
9388	0.0865	0.5	0.0872	0.6	0 0875	0.1
9366	0.1038	0.1	0 1038	0.2	0.1033	1.3
9268	0.1080	2.7	0.1089	2.2	0.1077	2.3
9405	0.1371	2.1	0.1367	3.0	0.1384	1.6
Sum	0.6909	11.0	0.6906	9.5	0.6902	16.7

SUMMARY

Silica dishes are satisfactory for use in the official method for the determination of potash. Slightly higher results were obtained in silica than in platinum dishes. New silica dishes were used in this project and have since been used in routine analyses. Approximately twenty-five determinations have been made in each of twelve dishes, and no deterioration is noticeable on the surface of any of the dishes. A larger insoluble residue was left after the washing out of the K_2PtCl_6 salt than was found with the former official method. This residue was usually increased when organic material was present in the fertilizer. The residue was not affected by re-washing with the reagents used in the method.

Very little difference was noted in the results obtained with the three methods of ignition studied.

DETERMINATION OF UREA NITROGEN IN FERTILIZER MIXTURES

By J. Y. Yee and R. O. E. Davis (U. S. Bureau of Chemistry and Soils, Washington, D. C.)

Urea nitrogen is defined in *Methods of Analysis, A.O.A.C.*, 1935, but no method is presented for its determination. This paper describes the application of such a method, developed by the writers,[1] to the analysis of mixed fertilizers.

Briefly the method consists of (1) digesting the sample with urease for 20 minutes at 40°C. to convert the urea to ammonium carbonate; (2) distilling for 20 minutes at the same temperature under reduced pressure with aeration after adding 20 cc. (or more, depending on the amount of sample taken) alcoholic solution of sodium hydroxide (4 grams per liter of methyl alcohol), and (3) absorbing the ammonia in standard acid. This method has been used successfully with samples containing only a few milligrams to as much as 0.2 gram of urea.

Slightly low results were obtained for urea in fertilizers containing fish meal, but this difficulty was overcome by analyzing the leachate of the samples instead of the solids. In this manner accurate and consistent results for urea in such mixtures were obtained. The following procedure was adopted for determining urea in mixed fertilizers:

Place 3–5 grams of sample on a filter paper (11 cm.) and wash with successive small portions of water, allowing each portion to pass through before adding more, until the filtrate measures about 95 cc. Make up the solution to 100 cc. and take 10 cc. aliquots for analysis.

Since, according to Armstrong and Horton,[2] urease reacts so specifically

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[1] *Ind. Eng. Chem. Anal. Ed.*, 7, 259 (1935).
[2] *Proc. Roy. Soc., London*, (B) 85, 109–27 (1913).

with urea, nitrogenous materials other than ammonium salts and compounds that liberate ammonia readily in alkaline solutions, such as formamide, do not interfere with the reaction. For samples containing the latter types of compounds, ammoniacal nitrogen is determined separately, but the same procedure is used except that no urease is added. Distillation at 40°C. in alcoholic solution does not decompose any of the urea in the sample. Urea nitrogen in such cases is determined by difference between the value for ammoniacal nitrogen and that for the combined urea plus ammoniacal nitrogen.

To test the applicability of the method for urea determination in fertilizers, six mixtures containing a number of fertilizer materials were prepared (Table 1). Before being mixed, all the materials except urea were

TABLE 1.—*Composition of fertilizer mixtures*

MATERIALS	MIXTURES*					
	A	B	C	D	E	F
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
$(NH_4)_2SO_4$ (21.11% N)	4 945	0.6202	—	5.1369	6 1147	—
Cottonseed meal	16.173	—	—	—	—	—
$CaCN_2$	--	0.5968	—	—	1 2698	3.0997
Milorganite	—	—	5 0250	—	—	—
$NaNO_3$	—	0 7959	5.7937	—	1 6935	4.1240
Starfish meal	—	—	—	7 0888	—	—
KCl	5.500	—	1.0070	—	—	2.1558
K_2SO_4	—	0.9702	1.1912	—	2 0643	—
K_2CO_3	—	—	—	2.1182	—	—
Dried blood	—	0.9259	—	—	1.9702	—
Superphosphate	43 381	—	—	18.0742	—	17.0016
Double phosphate	—	2.1668	—	—	4.6107	—
Am. double phosphate	—	—	5.3045	—	—	—
Phosphate rock	9.917	6.4408	12 0535	2 1504	17.5018	6.1352
Urea (45.74% N)	6.189†	1.5760‡	1.3889	1 5806	1 1532	1.4865

* 6–8–4 mixtures except E, 8–8–4.
† Powdered urea (46 31% N).
‡ Powdered urea (45.46% N).

ground to pass an 80-mesh screen to insure uniformity. Because powdered urea cakes readily, it was found later that a coarser (48–80-mesh) sample was more satisfactory. In each case all the materials except the ammonium salt and urea were thoroughly mixed, first on a piece of glazed paper and then in a bottle containing a few steel balls. After blank determinations for ammoniacal and urea nitrogen had been made on these mixtures, definite quantities of ammonium sulfate and urea were added to each and thoroughly mixed. Urea and ammoniacal nitrogen in these mixtures were then determined.

In addition to the six samples mentioned previously, eight others were

TABLE 2.—*Comparison of results*

MIXTURE	NH_3-N		UREA-N	
	ADDED	FOUND	ADDED	FOUND
	per cent	*per cent*	*per cent*	*per cent*
A	1.21	1.21	3.32	3.25
		1.20		3.25
B	0.93	0.92	5 08	5.08
		0.94		5.06
C	1.29	1.28	2 0	1.98
		1.29		1.99
D	2.99	2.97	2.0	1.96
		2.99		1.99
E	3.54	3.52	1.45	1.43
		3.51		1.43
F			2.0	1.94
				2.01
C-1		2.69		0.62
		2.72		0.60
		2.74		0.60
		2.71		0.63
C-2		3.04		0.42
		3.05		0.41
		3.04		0.43
		3.07		0.41
C-3		2.90		0.43
		2.92		0.42
		2.97		0.43
		2.94		0.42
C-1A			1.60	1.61
				1.57
C-2A			1.41	1.41
				1.39
C-3A			1.42	1.44
				1.42
W-1	3.09	3.17	1.22	1.20
W-2	6.26	6.25	2.44	2.40

analyzed; two of these were fertilizer mixtures (W-1 and W-2) prepared by C. W. Whittaker, and three were commercial samples (C-1, C-2, and C-3). Since the theoretical values for the urea and ammoniacal nitrogen in the commercial samples were not known, several determinations of

each were made to see how well the results could be duplicated. After these determinations had been made, a known quantity of urea was added to each of the three commercial samples, and urea nitrogen was again determined in the new mixtures (C-1A, C-2A, and C-3A).

The results of all these determinations (Table 2) check very closely with the theoretical values, showing that this method works satisfactorily with fertilizer mixtures.

SUMMARY

1. To determine the applicability of the Yee-Davis urease method for the determination of urea in mixed fertilizers, six mixtures were prepared from a variety of nitrogen carriers and other fertilizer materials. These and eight other mixtures were analyzed by the method.

2. Most of the nitrogen carriers do not affect the determination of urea, but in some cases where fish meal is present, better results are obtained by leaching the sample of fertilizer and making the ammoniacal nitrogen and urea determinations on the leachate. This procedure is recommended for all fertilizer mixtures.

3. The results obtained show close agreement between the quantities of urea added and those found, and excellent checks were obtained on three commercial fertilizer mixtures containing less than 0.5 per cent urea.

DETERMINATION OF ISOPROPYL ALCOHOL BY IMMERSION REFRACTOMETER

By J. Batscha and S. Reznek (U. S. Food and Drug Administration, New York, N. Y.)

The isopropyl alcohol content of dilute isopropyl alcohol-water mixtures may be readily determined with the Zeiss immersion refractometer. While there exist data on the refractive indices of such mixtures, the only table of immersion refractometer readings found in the literature is that in tl. book, "Alcohol, Its Production, Properties, and Industrial Applications."[1] The readings are at one per cent intervals for the single temperature 15.6°C. Adams and Nicholls[2] apply Simmonds' data to various alcohols, including isopropyl alcohol, but it is necessary to use two tables to convert immersion refractometer readings into per cent by volume of isopropyl alcohol. These tables also are intended for use at the single temperature, 15.6°C.

From the standpoint of convenience and completeness, however, these tables are obviously limited. Therefore, in view of the growing industrial and pharmaceutical use of isopropyl alcohol and the more convenient

[1] C. Simonds. Mac Millan (1919).
[2] *Analyst*, 54, 2 (1929).

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25°C.**

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
13.2						0.00
.4						0.14
.6					0.10	0.25
.8				0.08	0.22	0.36
14.0			0.05	0.20	0.34	0.48
.2		0.03	0.16	0.30	0.45	0.60
.4	0.00	0.14	0.28	0.42	0.56	0.71
.6	0.12	0.26	0.40	0.54	0.68	0.82
.8	0.24	0.38	0.52	0.66	0.80	0.94
15.0	0.36	0.50	0.64	0.78	0.92	1.06
.2	0.48	0.62	0.76	0.90	1.04	1.18
.4	0.60	0.74	0.87	1.01	1.14	1.28
.6	0.70	0.84	0.98	1.12	1.26	1.41
.8	0.80	0.94	1.09	1.23	1.38	1.52
16.0	0.92	1.06	1.21	1.35	1.50	1.64
.2	1.04	1.18	1.32	1 46	1.60	1.75
.4	1.14	1.28	1.43	1.57	1 72	1.87
.6	1.26	1.40	1.55	1.70	1 85	2.00
.8	1.38	1.52	1.66	1.80	1.95	2.10
17.0	1.50	1.64	1.78	1.92	2 06	2.20
.2	1.60	1.75	1.90	2.03	2.17	2.32
.4	1.72	1.86	2.01	2 15	2 30	2.45
.6	1.82	1.97	2.12	2.27	2.42	2.57
.8	1.94	2.08	2.23	2.37	2.52	2.68
18.0	2.06	2.20	2.36	2.50	2.65	2.80
.2	2.16	2.31	2.46	2.61	2.76	2.91
.4	2.28	2.43	2.58	2.73	2.88	3.04
.6	2.40	2.55	2.70	2.85	3.00	3.15
.8	2.50	2.65	2.80	2.95	3.10	3.26
19.0	2.62	2.77	2.92	3.07	3.22	3.38
.2	2.74	2.89	3 04	3.19	3.34	3.49
.4	2.84	2.99	3.15	3.30	3.46	3.61
.6	2.96	3.11	3.26	3.41	3.57	3.72
.8	3.08	3.23	3.38	3.53	3.68	3.84
20.0	3.18	3.33	3.48	3.63	3.79	3.95
.2	3.30	3.45	3.61	3.76	3.92	4.07
.4	3.42	3.57	3.72	3.87	4.02	4.18
.6	3.54	3.69	3.84	3.99	4.14	4.30
.8	3.64	3.79	3.95	4.10	4.26	4.42

* Calculated and arranged by J Batscha and S. Reznek, U. S. Food and Drug Administration, New York, N. Y.

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25°C.*—(*Continued*)

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
21.0	3.76	3.91	4.07	4.22	4.38	4.53
.2	3.88	4.03	4.18	4.33	4.48	4.64
.4	3.98	4.13	4 29	4.44	4.60	4.76
.6	4.10	4.25	4.41	4.56	4 72	4 88
.8	4 20	4.36	4.52	4.68	4.84	5.00
22.0	4.32	4.47	4.63	4.78	4.94	5.10
.2	4.44	4.59	4.74	4.89	5.04	5.20
.4	4.56	4.71	4.86	5.01	5.16	5.30
.6	4.66	4.81	4.96	5.11	5.26	5.42
.8	4.78	4.93	5.08	5.22	5.38	5.52
23.0	4.90	5 04	5.18	5.32	5.48	5.62
.2	5.00	5 14	5.29	5.43	5.58	5.72
.4	5.10	5.24	5.39	5.53	5.68	5.83
.6	5.22	5.36	5.51	5.65	5.80	5.94
.8	5.32	5.46	5.61	5.75	5.90	6.05
24.0	5.44	5.58	5.72	5.86	6.00	6.15
.2	5.54	5.68	5.83	5.97	6.12	6.26
.4	5.64	5.78	5 93	6 07	6 22	6.36
.6	5.75	5.89	6.04	6 18	6.32	6.46
.8	5.86	6.00	6.14	6.28	6.42	6.57
25.0	5.96	6.10	6.25	6.39	6.54	6.68
.2	6 06	6.20	6.35	6.49	6.64	6.78
.4	6.18	6.32	6.46	6.60	6.74	6.88
.6	6.29	6 43	6.57	6.71	6 85	6.99
.8	6.39	6 53	6.67	6.81	6.95	7.10
26.0	6.50	6.64	6.78	6.92	7.06	7.20
.2	6.60	6.74	6.88	7.02	7.16	7.30
.4	6.70	6.84	6.98	7.12	7.26	7.41
.6	6 81	6.95	7.09	7.23	7.37	7 52
.8	6.91	7.05	7.19	7.33	7.48	7.63
27.0	7.02	7.16	7.30	7.44	7.59	7 73
.2	7.12	7.26	7.40	7 54	7.69	7.83
.4	7.22	7.36	7.51	7 65	7.80	7.94
.6	7.32	7.46	7.61	7.75	7.90	8.04
.8	7.43	7.58	7.72	7.86	8.02	8 16
28.0	7.53	7.68	7.82	7.96	8 12	8.26
.2	7.64	7.78	7.93	8 07	8.22	8.36
.4	7.74	7.88	8.03	8 17	8.32	8.47
.6	7.85	7.99	8.14	8 28	8.43	8 57
.8	7.95	8.09	8.24	8.38	8.53	8.68

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25°C.*—(*Continued*)

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
29.0	8.06	8.20	8.35	8.49	8.64	8.79
.2	8.16	8.30	8.45	8.59	8.74	8.89
.4	8.26	8.41	8.56	8.71	8.86	9.00
.6	8.38	8.52	8.67	8.81	8.96	9.10
.8	8.48	8.62	8.77	8.91	9.06	9.20
30.0	8.58	8.72	8.87	9.01	9.16	9.31
.2	8.68	8.82	8.97	9.11	9.26	9.42
.4	8.80	8.94	9.09	9.23	9.38	9.52
.6	8.90	9.04	9.19	9.33	9.48	9.62
.8	9.00	9.14	9.29	9.43	9.58	9.73
31.0	9.12	9.26	9.41	9.55	9.70	9.84
.2	9.22	9.37	9.51	9.65	9.80	9.94
.4	9.34	9.49	9.63	9.77	9.92	10.06
.6	9.44	9.59	9.73	9.87	10.02	10.17
.8	9.56	9.70	9.84	9.98	10.12	10.26
32.0	9.66	9.80	9.94	10.08	10.22	10.36
.2	9.77	9.91	10.05	10.19	10.32	10.46
.4	9.88	10.01	10.15	10.29	10.43	10.56
.6	10.00	10.13	10.26	10.39	10.52	10.65
.8	10.12	10.24	10.37	10.49	10.62	10.75
33.0	10.21	10.34	10.47	10.60	10.73	10.86
.2	10.30	10.43	10.56	10.69	10.82	10.95
.4	10.40	10.53	10.66	10.79	10.92	11.05
.6	10.50	10.63	10.76	10.88	11.01	11.14
.8	10.59	10.72	10.85	10.98	11.11	11.25
34.0	10.68	10.81	10.95	11.08	11.22	11.35
.2	10.78	10.91	11.05	11.18	11.32	11.45
.4	10.87	11.00	11.14	11.27	11.41	11.54
.6	10.97	11.10	11.23	11.36	11.50	11.64
.8	11.06	11.19	11.33	11.46	11.60	11.74
35.0	11.15	11.29	11.43	11.57	11.71	11.84
.2	11.24	11.38	11.52	11.66	11.80	11.93
.4	11.34	11.48	11.62	11.76	11.90	12.03
.6	11.44	11.57	11.71	11.85	11.99	12.12
.8	11.54	11.67	11.81	11.94	12.08	12.22
36.0	11.64	11.77	11.91	12.04	12.18	12.32
.2	11.74	11.87	12.01	12.14	12.28	12.42
.4	11.83	11.97	12.11	12.24	12.38	12.52
.6	11.92	12.06	12.20	12.34	12.48	12.62
.8	12.02	12.16	12.30	12.44	12.58	12.72

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25° C.*—(*Continued*)

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
37.0	12.12	12.26	12.40	12.54	12.68	12.82
.2	12.21	12.36	12.50	12.64	12.78	12.92
.4	12.31	12.45	12.59	12.73	12.87	13.01
.6	12.40	12.54	12.68	12.82	12.96	13.11
.8	12.50	12.64	12.78	12.92	13.06	13.21
38.0	12.60	12.74	12.88	13.02	13.16	13.31
.2	12.69	12.83	12.98	13.12	13.27	13.41
.4	12.78	12.92	13.07	13.21	13.36	13.51
.6	12.88	13.02	13.17	13.31	13.46	13.60
.8	12.98	13.12	13.27	13.41	13.56	13.70
39.0	13.08	13.22	13.37	13.51	13.66	13.80
.2	13.18	13.32	13.47	13.61	13.76	13.90
.4	13.28	13.42	13.57	13.71	13.86	14.00
.6	13.37	13.52	13.67	13.81	13.96	14.10
.8	13.47	13.62	13.77	13.91	14.06	14.20
40.0	13.56	13.71	13.86	14.00	14.15	14.30
.2	13.65	13.80	13.96	14.11	14.26	14.40
.4	13.74	13.90	14.05	14.20	14.35	14.50
.6	13.84	14.01	14.16	14.31	14.46	14.60
.8	13.94	14.11	14.26	14.41	14.56	14.70
41.0	14.05	14.20	14.35	14.50	14.65	14.80
.2	14.14	14.29	14.44	14.59	14.74	14.89
.4	14.23	14.38	14.53	14.68	14.83	14.99
.6	14.32	14.47	14.63	14.78	14.94	15.09
.8	14.42	14.57	14.72	14.87	15.03	15.19
42.0	14.52	14.67	14.83	14.98	15.14	15.29
.2	14.61	14.76	14.92	15.07	15.23	15.38
.4	14.72	14.87	15.02	15.17	15.32	15.48
.6	14.81	14.96	15.12	15.27	15.43	15.58
.8	14.90	15.05	15.21	15.38	15.52	15.68
43.0	15.00	15.15	15.31	15.48	15.62	15.78
.2	15.10	15.25	15.41	15.58	15.72	15.88
.4	15.20	15.35	15.51	15.68	15.82	15.97
.6	15.29	15.44	15.60	15.77	15.91	16.07
.8	15.39	15.54	15.70	15.87	16.01	16.17
44.0	15.48	15.64	15.80	15.96	16.11	16.27
.2	15.57	15.73	15.89	16.05	16.21	16.36
.4	15.67	15.83	15.99	16.15	16.31	16.46
.6	15.76	15.92	16.08	16.24	16.40	16.56
.8	15.86	16.02	16.18	16.34	16.50	16.66

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25° C.—(Continued)*

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
45.0	15.94	16.10	16.27	16.44	16.60	16.77
.2	16.04	16.20	16.37	16.53	16.69	16.86
.4	16.14	16.30	16.46	16.62	16.78	16.95
.6	16.23	16.39	16.55	16.71	16.87	17.04
.8	16.33	16.49	16.65	16.81	16.97	17.14
46.0	16.42	16.58	16.75	16.91	17.08	17.24
.2	16.52	16.68	16.85	17.01	17.18	17.34
.4	16.62	16.78	16.95	17.11	17.28	17.44
.6	16.72	16.88	17.04	17.20	17.37	17.53
.8	16.82	16.98	17.14	17.30	17.47	17.64
47.0	16.91	17.07	17.23	17.39	17.56	17.74
.2	17.00	17.16	17.32	17.48	17.65	17.83
.4	17.10	17.26	17.42	17.58	17.75	17.93
.6	17.20	17.36	17.52	17.68	17.85	18.02
.8	17.29	17.46	17.62	17.79	17.96	18.13
48.0	17.38	17.55	17.72	17.89	18.06	18.23
.2	17.48	17.65	17.82	17.99	18.16	18.33
.4	17.57	17.74	17.91	18.08	18.25	18.42
.6	17.67	17.84	18.01	18.18	18.35	18.52
.8	17.77	17.94	18.11	18 28	18.45	18.62
49.0	17.87	18.04	18 21	18.38	18.55	18.72
.2	17.96	18.13	18.30	18.47	18.64	18.81
.4	18.05	18.22	18.39	18.56	18.73	18.91
.6	18.14	18.31	18.48	18.65	18.82	19.00
.8	18.24	18.41	18.58	18.75	18.92	19.10
50.0	18.33	18.50	18.68	18 86	19.03	19.20
.2	18.43	18 60	18.78	18.95	19.13	19.30
.4	18.53	18.70	18.88	19.05	19.22	19.40
.6	18.62	18.79	18.97	19 14	19.32	19.50
.8	18.72	18.89	19.07	19.24	19.42	19.60
51.0	18.81	18.99	19.17	19.35	19.53	19.70
.2	18.91	19.09	19.27	19.45	19.63	19.80
.4	19.00	19.18	19.36	19.54	19.72	19.90
.6	19.10	19.28	19.46	19.64	19.82	20.00
.8	19.20	19.38	19.57	19.75	19.93	20.11
52.0	19.30	19.48	19.67	19.85	20.04	20.22
.2	19.39	19.58	19.77	19.96	20.15	20.33
.4	19.49	19.68	19.87	20.06	20.25	20.43
.6	19.58	19.77	19.96	20.15	20.34	20.53
.8	19.68	19.87	20.06	20.25	20.44	20.64

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25°C.*—(*Continued*)

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
53.0	19.77	19.96	20.16	20.35	20.55	20.74
.2	19.87	20.06	20.25	20.44	20.64	20.84
.4	19.97	20.16	20.36	20.55	20.74	20.94
.6	20.06	20.25	20.45	20.64	20.84	21.04
.8	20.16	20.35	20.55	20.74	20.94	21.14
54.0	20.25	20.45	20.65	20.85	21.05	21.25
.2	20.34	20.54	20.74	20.94	21.14	21.35
.4	20.44	20.64	20.84	21.04	21.24	21.45
.6	20.54	20.74	20.95	21.15	21.36	21.56
.8	20.64	20.84	21.05	21.25	21.46	21.66
55.0	20.73	20.93	21.14	21.34	21.55	21.76
.2	20.82	21.03	21.24	21.45	21.66	21.86
.4	20.92	21.13	21.34	21.55	21.76	21.97
.6	21.01	21.22	21.43	21.64	21.85	22.07
.8	21.10	21.31	21.53	21.74	21.96	22.18
56.0	21.20	21.41	21.63	21.84	22.06	22.28
.2	21.30	21.51	21.73	21.94	22.16	22.38
.4	21.40	21.62	21.84	22.06	22.28	22.49
.6	21.49	21.71	21.93	22.15	22.37	22.59
.8	21.59	21.81	22.03	22.25	22.47	22.69
57.0	21.69	21.91	22.13	22.35	22.57	22.79
.2	21.79	22.01	22.23	22.45	22.67	22.89
.4	21.87	22.09	22.32	22.54	22.77	23.00
.6	21.97	22.19	22.42	22.64	22.87	23.10
.8	22.06	22.29	22.52	22.75	22.98	23.21
58.0	22.17	22.40	22.63	22.86	23.09	23.32
.2	22.26	22.49	22.72	22.95	23.18	23.42
.4	22.36	22.59	22.82	23.05	23.28	23.52
.6	22.46	22.69	22.92	23.15	23.38	23.62
.8	22.55	22.78	23.02	23.25	23.49	23.73
59.0	22.65	22.88	23.12	23.35	23.59	23.83
.2	22.75	22.98	23.22	23.45	23.69	23.93
.4	22.85	23.09	23.33	23.57	23.81	24.04
.6	22.94	23.18	23.42	23.66	23.90	24.14
.8	23.04	23.28	23.52	23.76	24.00	24.24
60.0	23.14	23.38	23.62	23.86	24.10	24.35
.2	23.24	23.48	23.72	23.96	24.20	24.45
.4	23.33	23.57	23.82	24.06	24.31	24.55
.6	23.43	23.67	23.91	24.15	24.40	24.65
.8	23.52	23.76	24.01	24.26	24.50	24.75

1.—*Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss immersion refractometer readings at 20–25°C.*—(*Continued*)

SCALE READING	PER CENT BY VOLUME					
	20°	21°	22°	23°	24°	25°
61.0	23.62	23.87	24.12	24.37	24.62	24.86
.2	23.71	23.96	24.21	24.46	24.71	24.96
.4	23.80	24.05	24.30	24.55	24.80	25.06
.6	23.90	24.15	24.41	24.66	24.92	
.8	24.00	24.25	24.51	24.76	25.02	
62.0	24.10	24.36	24.62	24.88		
.2	24.20	24.46	24.72	24.98		
.4	24.29	24.55	24.81	25.07		
.6	24.38	24.64	24.90			
.8	24.48	24.74	25.01			
63.0	24.58	24.84				
.2	24.68	24.94				
.4	24.77	25.05				
.6	24.87					
.8	24.97					

form of the A.O.A.C. tables for ethyl alcohol, the writers have recognized the need for and compiled a similar table of refractometer readings for isopropyl alcohol. As a supplement to this table the specific gravities of the various prepared mixtures were also determined and arranged in tabular form.

PREPARATION OF MIXTURES OF ISOPROPYL ALCOHOL AND WATER

The starting material used was Eastman 98–99% grade isopropyl alcohol. This was refluxed with freshly ignited lime and distilled. The distillate was then kept for several days in contact with freshly dehydrated copper sulfate, decanted, and redistilled. This product was next treated with a freshly cut piece of sodium and distilled, the fraction boiling 82.3–82.6°C. being collected in a small glass-stoppered bottle through an adapter reaching well into the neck of the bottle. The specific gravity of this material was 0.7867 at 20/20°C.

The aqueous mixtures were prepared by pipetting the alcohol into 50 cc. freshly boiled distilled water in volumetric flasks, the tip of the pipet being held below the surface of the water and the mixture being rotated to dissipate the heat of solution before adjustment to final volume. This method of delivery was checked by weighing samples of isopropyl alcohol delivered into weighed flasks containing water and calculating the volume from the specific gravity of the alcohol. The

2.—*Table showing relation between specific gravity at 20/20°C. and 25/25°C. and isopropyl alcohol content of mixtures of isopropyl alcohol and water.**

SP. GR. 20/20°	PER CENT BY VOLUME	SP. GR. 25/25°	PER CENT BY VOLUME
0.9971	2	0.9970	2
0.9957	3	0.9957	3
0.9944	4	0.9943	4
0.9931	5	0.9929	5
0.9919	6	0.9917	6
0.9907	7	0.9905	7
0.9895	8	0.9892	8
0.9883	9	0.9880	9
0.9871	10	0.9868	10
0.9860	11	0.9857	11
0.9849	12	0.9845	12
0.9838	13	0.9834	13
0.9827	14	0.9822	14
0.9816	15	0.9811	15
0.9805	16	0.9799	16
0.9795	17	0.9787	17
0.9784	18	0.9775	18
0.9773	19	0.9763	19
0.9762	20	0.9752	20
0.9751	21	0.9740	21
0.9740	22	0.9728	22
0.9729	23	0.9716	23
0.9718	24	0.9704	24
0.9708	25	0.9692	25

* Calculated and arranged by J. Batscha and S. Reznek, U. S. Food and Drug Administration, New York, N. Y.

pipets and flasks used were B.S. calibrated to deliver or contain, respectively, the volume specified at 20°C. A water bath was kept at 20°C. within ±0.2°C. and used for controlling the temperature of the alcohol, receiving flasks, and final volume adjustment. Solutions of 100 cc. in volume, containing 2, 5, 10, 15, 20, and 25 cc. of isopropyl alcohol, respectively, were prepared.

The Zeiss immersion refractometer readings for each solution were taken at 20° and 25°C., these temperatures being controlled by a water bath kept to within ±0.2°C. of the above temperatures as read on a B.S. calibrated thermometer graduated in 0.2°C. intervals. As a check on the refractometer, its reading with freshly boiled distilled water was taken. This reading was 14.43, which corresponds closely with that given in the A.O.A.C. ethyl alcohol tables.

The specific gravities were determined on the same solutions by means of a 25 cc. pycnometer, the temperature here being that recorded on the pycnometer itself. These were run by both of the writers, who used different pycnometers and recently standardized weights in one instance.

A COMPARISON OF SEVERAL PHYSICAL METHODS FOR THE DETERMINATION OF THE ALCOHOL CONTENT OF WINE*

By M. A. JOSLYN, G. L. MARSH, and J. FESSLER† (Fruit Products Division, University of California, Berkeley, Calif.)

The alcohol content of wine is generally estimated from the measurement of some physical property, such as density, boiling point, index of refraction, or surface tension, which changes regularly with alcohol content (1), even though the volumetric dichromate oxidation procedure of Semichon and Flanzy (2) has been shown by Fabre and Bremond (3) to be quite accurate and rapid. Although the effect of substances other than alcohol on the physical properties of wine may be estimated to be the same for wines of the same type and allowance made for their presence in making an approximate determination of alcohol, it is necessary to separate the alcohol from wine by some process of distillation in making more accurate determinations. However, there is the danger of incomplete and imperfect separation of the alcohol from the wine by distillation. This was recognized in 1876 by Maumene (4), who pointed out the effects of distilling over the higher alcohols, esters, aldehydes, and volatile acids and the losses of alcohol by entrainment with bubbles of carbon dioxide that may be present. He suggested neutralization of the wine before distillation to correct for the errors caused largely by the presence of volatile acids in the distillate and losses of alcohol by carbon dioxide. Where the distillation of the neutralized wine introduced ammonia into the distillate he recommended re-distillation after acidification.

The two most widely used methods for determining alcohol content are the densimetric analysis and the more rapid boiling-point analysis. The determination of the alcohol content of wine from the specific gravity of the distillate, as determined by a pycnometer, usually is considered to be the most accurate. It is preferred by Windisch (5) and is the official American procedure (6). However, this method is subject to several errors of manipulation, such as those caused by the imperfect construction of the pycnometer, which permits evaporation during weighing; by variations in the weight of hygroscopic moisture present on the surface of the pycnometer; by other errors in weighing; and, more particularly, by fluctuations in the temperature of the distillate. Great care must be exercised in temperature control; and accuracy of a hundredth of a per cent requires a temperature adjustment of about 0.01° and weighing to an accuracy of 0.5 mg. for a 50 cc. pycnometer. A difference in temperature of 0.1° affects the result more than a difference in weight of 1 mg. A simple method of avoiding errors due to temperature fluctuation and those due

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to deposition of dew on the pycnometer was described recently by Fuerst (7). Another source of error lies in the particular alcoholometric tables used (8).

For more rapid densimetric determination of alcohol the distillate may be adjusted to temperature and its alcoholic content determined by the use of a carefully calibrated and tested hydrometer. To obtain accurate results the hydrometer should be clean, in thermal equilibrium with the liquid, and carefully used. Walter (9) and Lucköw (10) have shown recently that the determination of alcohol by the hydrometer on the distillate may be as accurate as by the use of a pycnometer. The determination of the alcohol by measuring the density of the distillate by an alcoholometer, having been first used by Gay-Lussac in 1823, modified by Duval, perfected by Salleron and by Maumene, was adopted as the official procedure in France. Semichon and Flanzy (11) have reported recently that different hydrometers, on the same distillate, vary by as much as 0.4 per cent alcohol by volume and that the discrepancies of the instruments vary at different points on the scale. They stress the advisabilty of periodically checking the hydrometer by comparative determinations either with the pycnometer or the dichromate titration.

Because of the relatively great effect of wine extract on the specific gravity of alcohol-water mixtures, and because of the fact that a variation of 1 part in the fourth figure of the specific gravity of wine distillates introduces an error of 0.1 per cent alcohol by volume, the alcohol must be distilled from the wine before the determination (9), (10). However, it was found by Dumas, Desains and Thenard (12) that, although substances like cream of tartar lower the apparent alcohol content of alcohol-water solutions when tested in the ebullioscope of Vidal and other substances like invert sugar increase it, in a completely fermented wine the substances are so well compensated that the boiling point of the dry wine corresponds to that of an alcohol-water solution of the same alcohol content. Upon this principle is based the wide use in wineries of ebullioscopes for the rapid, approximate determination of the alcohol content of wine. The original ebullioscopes of Tabarie and of Vidal, who in 1829 were the first to make use of the principle that the boiling point of alcohol-water solutions decreases with increase in alcohol content, have been abandoned in favor of the instruments perfected by Malligand, Salleron and Amagat. The Salleron-Dujardin ebullioscope and Juerst's ebulliometer are the more commonly used instruments of this type in California (13).

Contrary to the findings of the committee of the French Academy of Science, Salleron found Malligand's scale too low when used with pure alcohol-water solutions and too high for wines. He developed the special Salleron ebulliometer and the sliding scale, indicating on one side the alcohol content of dry wine and on the other that of pure alcohol-water

solutions for a given boiling point. As pointed out by Thudichum and Dupré (1) the instrument does not exactly measure the boiling point as the temperature indicated is that of the liquid and not the temperature at which vapor and liquid are in equilibrium. The ebullioscopes were originally developed for the determination of the alcohol content of a completely fermented wine of low extract content or of the distillate obtained from it. None of the various types of ebullioscopes were developed for use with wines of high sugar and high alcohol content. However, their use for the latter practice is quite common in California, even by Federal gagers, and it may lead to serious errors.

The results of an investigation of the sources of errors in the ebullioscopic determination of the alcohol content of sweet wines and of a comparative study of several methods of determining alcohol are given in this paper.

EFFECT OF EXTRACT CONTENT ON THE INDICATIONS OF THE EBULLIOSCOPE

Since the ebullioscope is a highly empirical instrument it should be used only under the conditions for which it was calibrated. Careful attention should be given to such factors as the type of burner, size of flame, volume of wine used, and calibration with water at sufficiently frequent intervals to correct for fluctuations in barometric pressure. Niehaus (14) also has found that the method of heating and boiling and the position of the flame affect the determination. The writers find the gas microburner to be superior to the alcohol flame because it is easier to obtain a more uniform and reproducible heating condition with it. The thermometer is the most important part of the ebullioscope, and should be periodically tested against standard thermometers. The position of the thermometer in the boiling chamber is also important. Thus with a Dujardin-Salleron instrument, the thermometer in the regular position with half the bulb immersed in water read 100.20°C., but when it was raised so that all of the bulb was in vapor it read 100.10°C.

TABLE 1.—*Alcohol indications of the Dujardin-Salleron scale*
(Per cent by volume)

TEMPERATURE	MALLIGAND	VIN ORDINAIRE	TRUE*
°C			
87	—	23.0	—
88	20.5	19.8	22.0
89	17.6	17.2	18.2
90	14.9	14.7	16.7
91	12.8	12.6	14.2
92	10.8	10.7	12.0

* The concentration of alcohol in an alcohol-water solution having the boiling point indicated from the data by Noyes and Warfel, *J. Am. Chem. Soc.*, 23, 463 (1901).

It is well known that the addition of sugar to a solution of alcohol in water decreases the boiling point of the mixture and thus gives an apparent alcohol content higher than the actual. The dry wine scale of the Dujardin-Salleron ebullioscope reads lower for the same boiling point than the alcohol distillate scale (Table 1).

Thudichum and Dupré (1) report that the boiling point of alcohol-water solutions is a function of the proportion existing between the alcohol and water, regardless of other constituents. According to them a mixture that showed 20.05 per cent of alcohol by volume by ebullioscope and to which was added 5.95 per cent of sugar still showed 20.05 per cent, whereas there was in reality only 19.27 per cent of alcohol. Again, a

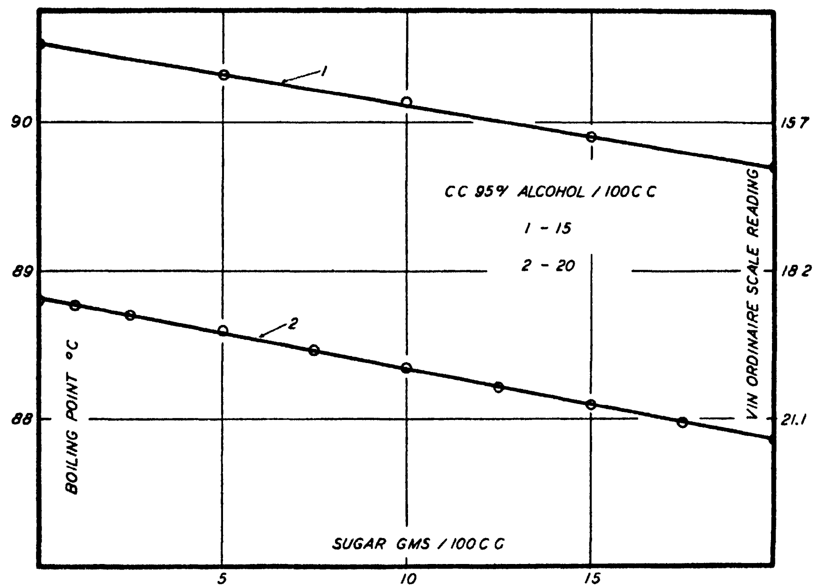

FIG. 1

spirit of 10 per cent by weight boils sensibly at the same temperature even after 10 or 20 per cent of sugar has been added, though in the last stage the alcohol is only 8.3 per cent instead of 10.0 per cent. The boiling points of a solution of 15 cc. and 20 cc. of 95 per cent alcohol, to which were added varying amounts of sugar, after dilution to 100 cc. are shown in Fig. 1. It is interesting to note that the alcohol content of the stronger solution is the same on the wine scale at 5 grams of sugar per 100 cc. as on the alcohol scale at 0 per cent. In the more dilute solution this is true at only 3.0 grams of sugar per 100 cc.

In adapting the ebullioscope to wines of high extract content various modifications are used. Dumas, Desains and Thenard (12) reported that

TABLE 2.—*Effect of dilution on the ebullioscopic determination of the alcohol content of wine liqueurs**

WINE TYPE	PURE WINE	1 VOL. WINE TO 1 VOL. WATER	1 VOL. WINE TO 2 VOLS. WATER	1 VOL. WINE TO 3 VOLS. WATER
Marosson	14.20	12.66	12.30	12.24
Moscatel de Sethubal	19.60	18.00	17.55	17.60
Malaga	20.45	18.70	18.36	18.20
Teneriffe	20.50	20.00	20.10	19.96
Frontignan muscat	14.00	12.80	12.69	12.72
Vermouth	18.20	17.60	17.60	17.60

* Data obtained by Dumas, Desains and Thenard in 1875.

by dilution of the wine with water a point would finally be reached at which the actual alcohol content would be obtained. Their data are shown in Table 2. Bertin (15) found that the presence of less than 10 grams of

TABLE 3.—*Effect of extract in wines of high alcohol content on the determination of alcohol by ebullioscope*

TYPE	BALLING	EXTRACT G./100 CC.	ALCOHOL, % BY VOLUME 1*	2*	3*	DIFFERENCE
Sherry	−2.8	4.1	21.4	—	21.60	−0.20
Sherry	−2.2	4.0	20.0	—	19.97	−0.03
Sherry	−0.4	5.8	18.8	--	18.72	+0.08
Sherry	1.0	7.5	21.4	20.5	—	+0.90
Muscatel	5.0	12.2	21.9	20.6	—	+1.30
Muscatel	5.6	12.8	20.9	20.3	—	+0.60
Port	5.8	12.7	22.8	—	21.61	+1.19
Port	6.2	13.4	21.5	20.5	—	+1.00
Port	6.4	12.9	21.5	19.6	—	+1.90
Muscatel	6.4	13.0	22.2	20.0	—	+2.20
Angelica	6 7	13.2	20.8	19.5	19.45	+1.35
Port	6.7	13.2	21.2	20.3	—	+0.90
Port	6.7	13.6	21.7	20.5	—	+1.20
Angelica	6 8	13.3	22.5	19.8	—	+2.70
Port	7.0	13.0	18.9	—	17.75	+1.15
Port	7.0	13.4	22.2	20.0	—	+2.20
Angelica	7 3	14.8	22.3	20.4	—	+1.90
Muscatel	8.4	15.2	21.4	20.0	—	+1.40
Port	8.8	14.6	19.5	19.0	—	+0.50
Muscatel	8.8	15.6	22.4	20.6	—	+1.80
Angelica	9.4	15.6	20.9	20.0	—	+0.90
Muscatel	9.4	15.9	21.6	20.4	—	+1.20
Muscatel	9 6	15.8	22.3	20.4	—	+1.90
Muscatel	9.6	16.6	21.6	20.6	—	+1.00
Port	9.8	16.0	21.0	20.4	20.10	+0.85
Port	10.6	18.0	22.9	20.0	—	+2.90
Angelica	11.2	18.4	21.2	19.5	—	+1.70
Angelica	13.0	20.2	22.4	20.1	20.20	+2.30

* (1) By ebullioscope on wine, (2) by hydrometer on distillate, (3) by pycnometer on distillate.

extract per liter has no effect on the ebullioscopic determination. Above 10 grams per liter the boiling point is lowered, so that the apparent alcohol content is higher than the true alcohol content, the difference being approximately 0.05 per cent for each 10 grams per liter. He suggests that 0.05 per cent be subtracted from the apparent alcohol content for each 10 grams of extract per liter and considers this procedure more accurate than dilution. On the other hand, Lherme (16) found no relation between the composition of the wine and difference of the results for alcohol content of 50 white wines as determined by the Malligand ebullioscope and by the density of the distillate.

TABLE 4.—*Effect of dilution of high extract wines on alcohol determination by ebullioscope*

TYPE	EXTRACT	ALCOHOL, % BY VOLUME NO DILUTION	1 WINE 1 WATER	1 WINE 2 WATER	ON DISTILLATE
Sherry	4.0	20 0	20 0	—	20.0
Sherry	4.1	21.4	21.5	--	21.6
Sherry	5.8	18 8	18.7	—	18.7
Sherry	7.5	21.4		21.2	20.5
Muscatel	12.8	20.9		21.2	20.4
Port	12.7	22.8	21.5		21.6
Port	12 9	21.5	20.2		19.6
Port	13 0	18 8	18 0	17.8	18.0
Muscatel	13 0	22.2	21.2		20 0
Angelica	13 2	20.8	19.6		19.4
Angelica	13 3	22 5	21.5		19.8
Muscatel	15 2	21.4		20.9	19 0
Muscatel	15.8	22.3		21 3	20.2
Angelica	19 6	23 0	21.1		19 0
Angelica	20 2	22 4		21.0	20 2

The effect of extract on the apparent alcohol content of California wines is shown in Table 3. It is seen that up to an extract content of 5.8 there is little difference between the apparent and true alcohol content, but that this increases with extract content, although not in an entirely regular manner.

The effect of dilution is shown in Table 4. For approximate results a 1:1 dilution is sufficient.

It has been pointed out by Filandeau (17) that although the ebullioscope gives an alcohol content with sufficient accuracy for common use it is necessary to check the instruments. Ebullioscopes actually in service were found to be in error owing to defects in construction as well as errors in manipulation. The variations in alcohol content as determined by several collaborators are shown in Table 5. Data obtained by the writers on three different ebullioscopes on sweet wine distillates (Table 6) also indi-

cate that the differences between instruments may be appreciable even when they are tested by the same person under like conditions.

INDIRECT DETERMINATION OF ALCOHOL

The estimation of the alcohol and extract content from the refractive index and specific gravity measurements was applied to beer by Race(18),

TABLE 5.—*Collaborative data on the determination of the alcohol content of wine by the ebullioscope (after Filandeau)*

ESTABLISHMENT AND INSTRUMENT	RED WINE 1	RED WINE 2	RED WINE 3	WHITE WINE 1
By distillation				
Laboratory A	8.2	9.5	12.6	•9.8
B	8.25	9.4	12.45	9.75
C	8.2	9.4	12.7	9.6
By ebullioscope				
B_1 Petit Malligand	8.3	9.6	12.5	9.8
B_2 Grand Malligand	8 4	9.8	12.7	10.3
D Ebulliometre	8.2	9.4	12.3	9.9
Grand Malligand	8.2	9 4	12.4	9.9
Grand Malligand	8.5	9.9	12.9	10.3
Alambic Salleron	8.5	9.3	12.0	10 0
F Petit Malligand	8 5	9.5	12.7	10.1
G Petit Malligand	8.4	9 6	12.8	10 1
Petit Malligand	8.4	9.6	12.7	10.1
Ebulliometre	8.5	9.7	12.7	10.1
J Petit Malligand	8.3	9.8	12.9	10.2
L Petit Malligand	8.1	9.5	12.6	10.0
V Ebulliometre	8.3	9.5	12.5	10.0
O Grand Malligand	8.7	9.8	12.9	10.4
B_3 Petit Malligand	8.2	9.5	12.6	9.9
Grand Malligand	8.4	9.7	12.7	10.1
Lab. A Petit Malligand	8.2	9.6	12.8	9.9
Petit Malligand	8.3	9.5	12.6	9.9
Petit Malligand	8.4	9.5	12.7	10.0
Lab. B Ebulliometre S	8.3	9.4	12.5	9.9
M	8.4	9.5	12.7	10.0
S	8.3	9.45	12.45	9.95
M	8.4	9.55	12.7	10.1
Lab. C. Malligand	8.4	9 5	12.6	9.9

to brandy by Saar (19) and Beckel (20), and to wine by Newton and Munro (21). It has been shown recently that the results obtained by the Newton and Munro method were very different from the results obtained by Prélat and Mendivelzúa by the usual method for Argentine wines (25) and that the alcohol content of a California dry red wine was lower by less than 0.2 per cent by the Newton and Munro procedure but about 0.4–0.5 per cent for dry white wine (26). The data shown in Table 7 indi-

TABLE 6.—*Comparison of ebullioscopes*

TYPE OF WINE	PER CENT ALCOHOL BY VOLUME ON DISTILLATE			
	BY EBULLIOSCOPE 1	BY EBULLIOSCOPE 2	BY EBULLIOSCOPE 3	BY HYDROMETER
Port	20.6	19.5	20.3	20.4
Port	19.9	19.8	19.2	20.3
Port	19.8	19.9	20.7	20.0
Port	20.6	19.9	20 9	20 0
Port	18.2	18.6	20.1	19 0
Port	20.0	19.9	20 3	20.5
Port	19.7	20.1	20.9	20.5
Muscatel	20.8	19.9	20.3	20.4
Muscatel	20.3	19.9	20.2	20.3
Muscatel	19.0	20.0	20.7	20.4
Muscatel	19.2	20.3	20.9	20.6
Muscatel	19 2	20.4	20 9	20.6
Muscatel	19.3	18.9	19 2	20.0
Angelica	18 9	19 4	19.5	19.5
Angelica	19.4	19 9	20.3	20.4
Angelica	19.9	19.5	19.8	20.1
Angelica	19.3	19 5	20 6	20 0

cate that the indirect determination of alcohol by the Newton and Munro procedure is fairly satisfactory for red wines but not for white.

RESULTS OF COLLABORATIVE DETERMINATION OF ALCOHOL

To test the reliability and reproducibility of current methods of wine analyses a series of conferences on methods of wine analysis and of collaborative tests of these methods was initiated.

TABLE 7.—*Indirect determination of alcohol by the Newton and Munro procedure*

WINE	EXTRACT	ALCOHOL BY PYCNOMETER	SPECIFIC GRAVITY 20/20	REFRACTIVE INDEX	CALCULATED ALCOHOL	CALCULATED EXTRACT
	g./100 cc.	*% by volume*			*% by volume*	*g./100 cc.*
Dry white	1.96	12.15	0.9919	1.3419	11.42	1 37
Dry red	3.22	13.62	0.9951	1.3457	13 95	2.89
Sherry material	4.11	21.59	0.9893	1.3515	21.58	3 70
Port	12.65	21.61	1.0229	1.3632	23 73	8.91
Claret	—	13.33	0.99259	1.34406	13.13	2 1
Angelica	13 2	19 50	1.02871	1.36306	19.34	12 6

Discrepancies in analytical results obtained by different chemical laboratories for presumably the same wine are often embarrassing. They may be seriously so when wines are received by the consignee on a falling market. The variation of the results obtained by commercial analytical firms

from those reported by the winery's own chemist usually have been ascribed to the cruder and more rapid methods used by the winery chemist. Although the winery chemist often makes use of rapid control methods that are not so time consuming and complicated as the more accurate methods, their differences in reported results are much greater than can be ascribed to methods used. Errors due to sampling and poor technique are probably involved.

For many years collaborative tests of methods of alcohol determination have not been made, the last one being by Colby in 1896 (24). The results of this test of the pycnometric procedure are shown in Table 8. Several serious discrepancies occur in the results, but Colby considered the differences were admissible, and the results should be considered quite satisfactory. Some results obtained on several wines by commercial analytical

TABLE 8.—*Alcohol content of wine as determined by collaborators in 1895*
(Per cent by volume)

COLLABORATOR	WINE		
	VERDAL	ZINFANDEL	SHERRY
G. L. Teller	8.25	13.16	21.40
W. C. Blasdale	8.14	13.29	21.27
Geo. E. Colby	8.45	13.10	21.25
F. T. Bioletti	8.30	13.15	21.06
Marvin Curtes	8.29	13.29	21.37
H. J. Patterson	5.96	14.00	20.29
Charles L. Parsons	8.34	13.50	21.39
R. D. Hiltner	8.18	13.16	21.47
W. D. Bigelow	8.31	13.33	—
Slewenski	6.87	11.87	—

laboratories, State Board of Health laboratories, and the winery chemists are shown in Tables 9 and 10. The analyses of these results are shown in Tables 11 and 12. Apparently even the pycnometer results can only be considered as accurate and reproducible to within ± 0.1 per cent.

The results of the collaborators for the first wines may be criticized on the bases that the technique for using the ebullioscope was not uniform and standardized. The volume of water used in the blank run and the volume of wine may have been varied. Furthermore the ebullioscope thermometer may have been in error or improperly placed. Thus the Dujardin-Salleron ebullioscope is calibrated for use with 15 cc. of water and 50 cc. of wine. The effect of dilution and of varying the volume of wine used is shown in Table 13, which gives the boiling point and the apparent alcohol content of a sherry wine that contained 18.72 per cent alcohol by volume as determined by the pycnometer. Similar results were obtained for dry wines. Thus for a dry wine testing 12.32 per cent alcohol by pycnometer, the following apparent alcohol contents were obtained: 10 cc.

TABLE 9.—*Collaborative results on alcohol content of dry wine*
(Per cent by volume)

LABORATORY	EBULLIOSCOPE			HYDROMETER			PYCNOMETER		
	RED-1	RED-2	WHITE-1	RED-1	RED-2	WHITE-1	RED-1	RED-2	WHITE-1
Fruit Products	13 2	14.4	14 2				13 31	14.43	13.98
Viticulture	12.9	14.3	14 0	13.0	14.28	13.95	13.01	14.26	13.95
State	—	14.5	—	13.2	14 2				
Comm. 1	13.4	14.5	14.3				13 5	14.40	13.95
2	13.10	14.4	14 1				13 12	—	14.15
3	13.2	14.6		13 4			13.32		
4							13.04		
Treasury			14.35			14 30			
Comm. 5							13.38	14.27	
Hartmann									13.50
Winery 1	12.95	14.2							
2	13.3	14.5	14.3						
3	13.2	—	—				13.17		
4	13.4	14.2	14.0	13.31			13.33	14.17	13.81
5	13.2	14.5	14.3						
6	12.9	13.8	13.9						
7	13.6	14.3	14.4	13 27	14.2	13.5	13 34		
8	13.1	14.4	—	—	14 5				
9	13.2	14.3	14.5						
10	13.0	14.4	14.1						
11	13.1	14.2	14.0	—	—	14.2			
12	13.5	14.4	—						
13	13.4	14.3	—	13.1	14.24				
14	—	14.3	—	14.3				14.33	
15	—	14.6	—	—	14.34				
16	—	14.2	14.3						
17	—	—	13.6			13.8			
18	11 3	13.6	14.2	13.1	13.5				
19	—	—	14.1						
20	—	—	14.1						

TABLE 10.—*Collaborative results on alcohol content of sweet wine*
(Per cent by volume)

LABORATORY	EBULLIOSCOPE, DIRECT			EBULLIOSCOPE, 1+1			EBULLIOSCOPE, DISTILLATE			PYCNOMETER			HYDROMETER			REFRACTOMETER		
	ANGELICA	PORT	PORT SHERRY	ANGELICA	PORT	PORT SHERRY	ANGELICA	PORT	PORT SHERRY	ANGELICA	PORT	PORT SHERRY	ANGELICA	PORT	PORT SHERRY	ANGELICA	PORT	PORT SHERRY
Fruit Products	20.6	18.8	19.4	19.6	18.0	19.1	19.6	17.9	18.5	19.47	17.75	18.76						
Viticulture			19.0	19.3	17.5	18.9			18.7	19.43	17.55	18.73	19.42	17.56	18.77	19.47	17.75	18.9
State		17.9					18.0						19.6	18.0		19.47		
Comm. 1	19.6		19.5		17.52	18.94	19.7		18.64	19.65	17.55	18.8				19.65		18.8
2	20.6		19.15	19.8	18.0	19.1	19.2			19.47		19.0						
3	21.0			19.8	18.0					19.4			19.5	17.75				
4										19.41								
Treasury			19.49						19.23			19.00			19.25			
Comm. 5										19.55	17.73							
Hartmann												18.23						18.18
Winery																		
1				19.7	18.35													
2			19.4	19.9	18.3	19.3	19.3		18.8									
3				19.95			19.4			19.5								
4	19.8			19.5	17.8	19.0		17.5	18.3	19.49	17.58	18.85	19.82			19.41		
5			19.2	19.6	17.9	18.8			18.8									
6					17.5	18.4		17.2										
7	20.0	18.0	19.3			19.0		17.5	18.4	20.24			19.65	17.4	18.4			
8				19.5	18.1									18.2				
9				19.5	17.9	19.5		18.0	19.1						19.2			
10			19.1	19.4	17.4	18.8												
11			19.0		18.2	19.2			19.2				19.8		19.4			
12					17.8													
13	20.0	18.2			18.0			17.6					19.6	18.0				
14				17.7			17.6			17.84				17.4				
15					18.2		17.6							17.22				
16			19.4		18.1	18.8			18.8									
17			18.5			18.6			17.9				•		18.6			
18					16.6	18.4	16.4		18.25				19.0		18.2			
19			19.0		18.3	19.0		18.2	18.8				19.6					
20			19.15			18.96			18.5			18.90						

TABLE 11.—*Alcohol content of dry wine*

METHOD	COLLABORATORS REPORTING	AVERAGE	MAXIMUM	MINIMUM	NUMBER AGREEING WITHIN ±0.1%	±0.2%
			Wine 1			
Ebullioscope Direct	18	13.2	13.6	(11.3) 12.9	8	12
Ebullioscope Distillate	4	13.4	13.5	13 0	3	3
Hydrometer	10	13.3	13.7	13.0	5	8
Refractometer	3	13.3	13.5	13.1	None	None
Pycnometer	10	13.25	13.5	13.0	6	9
			Wine 2			
Ebullioscope Direct	23	14.3	14.6	13.6	13	18
Pycnometer	8	14.3	14.43	14.17	8	8
			Wine 3			
Ebullioscope Direct	18	14 2	14.5	13.6	10	14
Hydrometer	5	14.0	14.3	13.5	2	3
Pycnometer	6	13 89	14.15	13 5	4	4

TABLE 12.—*Alcohol content of sweet wine*

METHOD	COLLABORATORS REPORTING	AVERAGE	MAXIMUM	MINIMUM	NUMBER AGREEING WITHIN ±0.1%	±0.2%
			Wine 1			
Ebullioscope Direct	7	20.1	20.6	19 5	2	3
Ebullioscope Diluted wine 1:1	12	19.7	20.0	19.2	6	9
Ebullioscope Distillate	7	19 5	19.75	19.2	5	6
Hydrometer	10	19.6	19.9	19.0	4	9
Refractometer	3	19 5	19.4	19.65	3	3
Pycnometer	10	19.5	19.7	19.41	9	10
			Wine 2			
Ebullioscope Wine diluted 1:1	22	18.0	18.4	17.5	11	14
Pycnometer	8	17.64	17.5	17 84	6	8
			Wine 3			
Ebullioscope Direct	14	19.2	19.5	18.5	6	11
Ebullioscope Wine diluted 1:1	17	18.9	19.5	18.4	8	12
Ebullioscope Distillate	15	18.6	19.2	18.2	4	9
Hydrometer	7	18.8	19.2	18.2	1	1
Refractometer	8	18.53	19.0	18.23	0	4
Pycnometer	3	18.85*	18.9	(18.18)	2	2

* Weighted.

wine, 13.9; 25 cc. wine, 12.2; and 50 cc. wine, 12.3. When the bulb is at least half immersed, as it should be with 15 cc. of water in a properly constructed ebullioscope, varying the volume of water from 15 to 50 cc. has no effect on the boiling point. In some types of ebullioscopes a decrease of as much as 0.10°C. is noted and this may introduce an error of about

TABLE 13.—*Effect of dilution and of variation in volume of wine used on the indication of the ebullioscope*

TREATMENT	BOILING POINT	ALCOHOL CONTENT
	°C	*% by volume*
5 cc. of distillate*	98.31	18.5
10*	96.70	18.75
25*	92.85	18.8 •
50	88.60	18.8
5 cc. of wine*	98.25	18.7
10*	96.70	18.45
25*	92.80	18.74
50	88.35	18.9
5 cc. of wine	90.0	14.35
10	87.6	20.5
15	87.85	19.75
25	88.16	18.9
40	88.16	18.9
50	88.19	18.85

* Diluted to 50 cc.

0.2–0.4 per cent alcohol. The extent of the variation found by the collaborators is shown in Table 14. In making the subsequent analyses the ebullioscope technique was standardized for a 25 cc. water blank.

In general, the results indicate that with suitable precautions the alcohol content may be determined to ± 0.1 per cent by pycnometer and ± 0.2 per cent by ebullioscope. If the results do not fall into these ranges they are definitely in error.

LITERATURE CITED

(1) See for example: Thudichum and Dupré, A Treatise on the Origin, Nature, and Varieties of Wine, Chap. 5. Macmillan and Company, London (1872); Portes and Ruyssen, Traité de la Vigne, Vol. 2, pp. 540–7. Octave Doin, Paris (1888); Babo and Mach, Handbuch der Kellerwirtschaft, pp. 578–590. Paul Parey, Berlin (1910); Gayon and Laborde, Vins, pp.88–102. Librarie Polytechnique Ch. Beranger, Paris (1912); Jules Dujardin, Lucien Dujardin and Rene Dujardin, Notice sur les instruments de precision appliques a l'oenologie, pp. 307–440. Paris (1923).

(2) *Ann. fals.* **22**, 139–52 (1929).

(3) *IV Congr. intern. tech chim. ind. agr. Bruxelles*, **3**, 308–15 (1935).

(4) *Ann. chim. phys.* Ser. 5, **9**, 499–570 (1876).

TABLE 14.—*Comparison of ebullioscopes*

ANALYST	EBULLIOSCOPE	CONDITION	BOILING POINT WATER 15 cc.	BOILING POINT WATER 25 cc	ALCOHOL IN CLARET 15 cc.	ALCOHOL IN CLARET 25 cc.
			°C.	°C.		
B	B.K.H. Ebulliometer	Original thermom. and rubber stopper	99.68	99.68	14.5	14.5
	Tag Twin Ebulliometer	Original	—	—	14.5	—
C	Dujardin-Salleron	Original thermom. and stopper	100.17	100.20	14.3	14.4
	Dujardin-Salleron	Original thermom. and stopper	100.31	100.37	—	—
D	—	—	—	—	14.6	14.6
E	Braun Ebulliometer	Original	100.1	100.1	14.3	14.3
F	Dujardin-Salleron	Orig. thermom. but new stopper	100.03	100.12	14.2	14.4
		Orig. thermom. but new stopper	100.12	100.22		
G	Dujardin-Salleron	Orig. thermom. but new stopper	100.26	100.36	14.2	14.4
H	Salleron-Dujardin	Not orig. thermom. and stopper	—	—	14.4	14.5
I	E & A Juerst	New thermom. and stopper	99.29	99.35(50)	14.49	14.52
	Tag Twin Ebulliometer	Original	99.38(25)	99.40(50)	14.24	14.30
	Malligand		—	—	—	14.20(35)
J	B.K.H. Ebulliometer	Tag thermom., Salleron slide	—	—	—	14.4
K	Salleron-Dujardin	Original (1935)	100.08	100.08	14.2	14.2
L	Seul Vertible	New thermom. and stopper	99.7	99.8	13.8	13.9
M	Salleron-Dujardin	Original (1916?)	100.2	100.25	14.5	14.6
N	Salleron-Dujardin	New thermom. and stopper	100.7	100.7	13.6	13.6
O	Salleron-Dujardin	New thermom. and stopper	99.65–99.72	99.82–99.85	14.5	13.95–14.
P	Salleron-Dujardin	New thermom. and stopper	100.28	100.31	14.2	14.3
Q	Juerst	Not original thermom. or stopper	—	—	14.4	14.5
R	—	—	—	—	14.3	14.3
S	—	—	100.15	100.1	14.3	—
T	—	—	100.15	100.15	14.3	14.3
U	Salleron-Dujardin	Not original	99.75	100.0	14.15	14.2
V	Braun Ebulliometer	Not original but duplicate Tag thermometer	100.12	100.18	14.25	14.4
W	Salleron-Dujardin	Original	100.1	100.1	14.2	14.2

(5) Die Chemische Untersuchung und Beurtheilung des Weins, pp. 52–6. Berlin (1896).

(6) *Methods of Analysis, A.O.A.C.*, 1935, 163.

(7) *Ind. Eng. Chem. Anal. Ed.*, **2**, 30–1 (1930).

(8) For a discussion of alcholometric tables see: C. Mariller, *Bull. assoc. chim. sucr. dist.*, **46**, 497–504 (1929).

(9) Getränke Ind., 1927, 285–6; *Deut. Destillateur-Ztg.*, **55**, 583–4 (1934).

(10) *Brennerei-Ztg.*, **48**, 168 (1931); *Z. Spiritusind*, **58**, 279 (1935); *Mitt. Abt. Trinnkbranntw.*, Inst. Gär., **25**, No. 3, 31–2. Berlin (1935); *Brennerei-Ztg.*, **52**, 161 (1935).

(11) *Ann. fals.*, **26**, 92–3 (1934).

(12) *Compt. rend.*, **80**, 1114–33 (1875).

(13) Directions for their use are given in U. S. Treasury Dept., Bur. Industrial Alcohol Regulations No. 7, pp. 161–4.

(14) *S. African Wine Ann.*, Special Ed. of Wine and Spirits, **4**, 1693–7 (1934).

(15) *Ann. fals.*, **26**, 103–5 (1933).

(16) *Ibid.*, 546–52.

(17) *Ibid.*, **19**, 531–6 (1926).

(18) *J. Soc. Chem. Ind.*, **27**, 544 (1908).

(19) *Z. Untersuch. Lebensm.*, **56**, 144–58 (1928).

(20) *Ibid.*, **58**, 78–98 (1929).

(21) *Can. Chem. Met.*, **17**, 119–120 (1933).

(22) *Anales asoc. quim. argentina*, **22**, 76–7 (1934).

(23) *J. Assoc. Official Agr. Chemists*, **18**, 307–13 (1935).

(24) U. S. Dept. Agr. Bur. Chem. Bull. **47**, 95–7 (1896).

THE COLORIMETRIC DETERMINATION OF LACTIC ACID IN MILK AND MILK PRODUCTS

By FRED HILLIG (Food Division,* U. S. Food and Drug Administration, Washington, D. C.)

Of the many procedures for the determination of lactic acid, the aldehyde method has received the most attention from biologists as well as food chemists. It is preferred because it is simple to operate, but more particularly because it requires only small quantities of material.

The latest contribution to the determination of lactic acid in milk and milk products by the aldehyde method is that of Troy and Sharpe.[1] These authors point out that certain compounds, notably sucrose and glycerin, which may be present in a milk product and are not (or only partially) removed in the isolation procedure, yield aldehyde on oxidation with potassium permanganate. They have made a thorough study of all the phases of the procedure and have determined appropriate corrective factors for the interferences inherent in the method.

It has long been known that ferric chloride reacts with certain acids to form definite colors. Williams, Muller and Neiderl[2] have investigated

* W. B. White, Chief.
[1] Cornell Univ. Agr. Expt. Sta. Memoir 179, June, 1935.
[2] Mikrochemie, 9, 268 (1931).

this reaction for a variety of organic acids, but they have restricted their work to pure acids, and made no attempt to apply the principle to the determination of lactic acid in food products. However, their work suggested that it might be possible to utilize the yellow color produced by the reaction between lactic acid and ferric chloride in the determination of lactic acid in milk and milk products generally, and therefore the study here presented was undertaken.

The principal steps in the procedure are: (1) preparation of a serum, (2) extraction of the lactic acid, (3) purification of the extracted acid with carbon, and (4) development and final estimation of lactic acid colorimetrically.

Since a liquid extractor is used for the isolation of lactic acid, it is necessary to prepare a serum that will contain all the lactic acid present in the product under examination. It was found that the proteins could be most advantageously precipitated from a water solution with sulfuric and phosphotungstic acids, and that the filtrate could then be introduced into the extractor without any further manipulation. Although lactalbumin is not completely removed, the small quantity remaining is not sufficient to cause troublesome foaming in the extractor.

A type of continuous extractor described previously[1] is suitable for this purpose, but in order to accommodate 50 cc. of solution in the extractor, the following changes in dimensions were made:

	inches
Overall length jacket	22
Length inner tube	20
Distance from bottom to overflow	13
No changes in diameter of tubes.	

That these changes do not decrease the efficiency of the extractor, is shown by the fact that an extraction of 2 hours was sufficient to yield from 97 to 99 per cent of the lactic acid present when 50 cc. of solution was used.

Part of the citric acid normal to milk is extracted with the lactic acid, but its complete removal is necessary because citric acid forms a color similar to that produced by lactic acid when treated with ferric chloride. Removal is accomplished by a treatment with barium hydroxide in an alcoholic solution in which barium lactate is soluble.

The use of carbon is necessary, not so much for its decolorizing properties, but rather for its ability to adsorb the small quantities of material remaining in the solution after the removal of citric acid that produce a brownish interfering color when treated with ferric chloride. It is recognized that carbon will adsorb lactic acid, and it was found that under controlled conditions this adsorption is practically constant, amounting to about 7 per cent of the lactic acid present. This loss of lactic acid was

[1] *This Journal*, 16, 3, 435 (1933).

verified on numerous determinations and was found to vary only slightly. This is the only loss in the procedure and it is corrected in the standard curve and the preparation of the color standards. For the proper functioning of the carbon a slightly acid medium is necessary. A definite quantity of 0.1 *N* hydrochloric acid is added to the material for the carbon treatment, and in the subsequent development of the color with ferric chloride. To prevent adsorption of hydrochloric acid during the treatment, it is necessary to saturate the carbon with the hydrochloric acid. The carbon used in this work is known as "Nuchar W." Other carbons, namely, Suchar, Darko G60, and Carbex E were found to be suitable when treated in the manner described. Norite A and animal charcoal are not so satisfactory for this particular work since the adsorption of lactic acid is greater than with the other carbons.

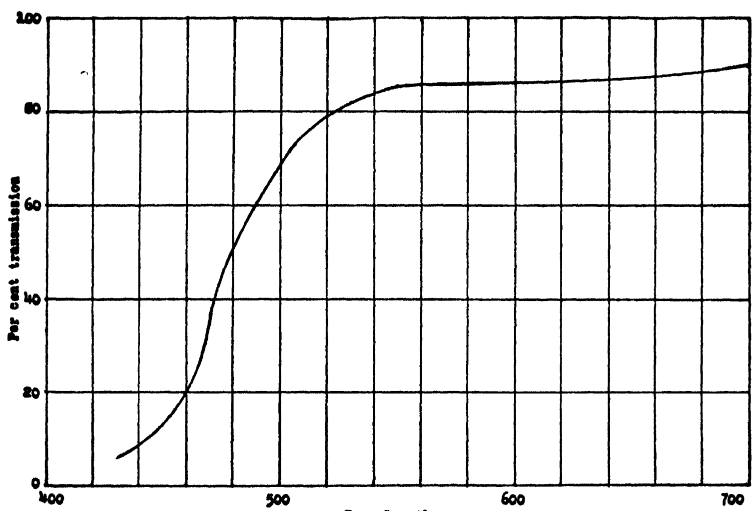

FIG. 1. TRANSMISSION THROUGH 1 INCH CELL OF LACTIC ACID-FERRIC CHLORIDE COMPLEX

Nessler tubes are satisfactory for making color comparisons, but when they are used it is necessary to give the standards a carbon treatment similar to that followed in the determination in order to compensate for the adsorption factor. This operation is rather tedious and time-consuming. The photometer gives more rapid and accurate readings, and consequently it was used in obtaining the data reported in this paper. The instrument selected* has been briefly described and illustrated in a paper relating to the dithizone methods for the determination of lead by Clifford and Wichmann.[1] It is a visual instrument with a comparator head, which

* The instrument will be more fully described in a forthcoming paper by Brice and Clifford.
[1] *This Journal*, 19, 130 (1936).

brings two beams of light together within an eyepiece, one beam passing through a photometric wedge made of Bausch & Lomb optical glass. The wedge is 15 cm. long and varies in thickness from 1 cm. at the thick end to less than 0.5 mm. at the thin end, and is compensated by a similar wedge of clear optical glass. The light intensities of the two beams are balanced by moving the wedge back and forth. As this wedge is neutral,

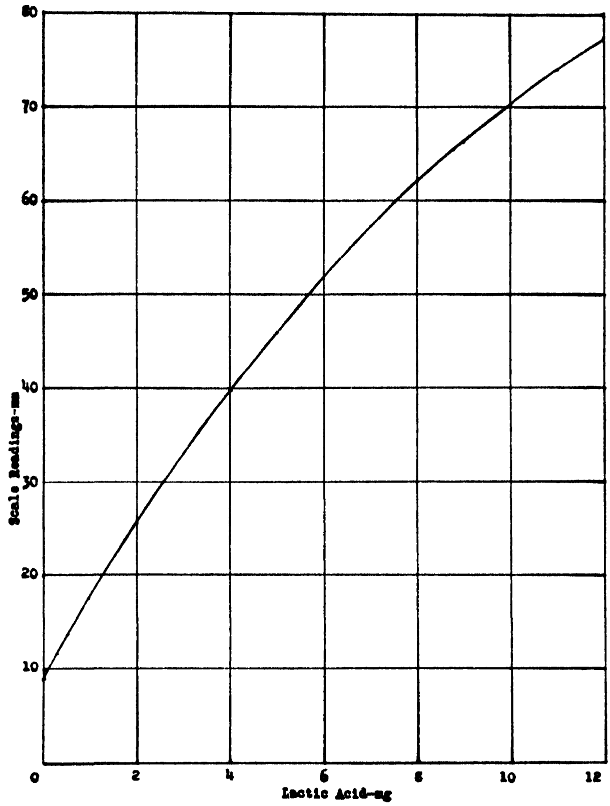

FIG. 2. STANDARD CURVE.

it should transmit light of all wave lengths equally well. Hence, it is possible to select a light filter that transmits only in that spectral region in which the solution being examined shows its strongest light absorption. This filter is mounted on the eyepiece, thus giving a mono-chromatic field.

The yellow color produced by the reaction between lactic acid and ferric chloride is represented by the spectro-transmission curve, Fig. 1, which gives the transmission through a one inch cell. The color shows increasing absorption in the blue and violet region of the spectrum.

The curve indicates that a filter centering around 450 mμ would be

satisfactory. In fact such a filter was used in the work. However, in order partially to eliminate hue difference, a filter of Noviol Yellow (Corning No. 038 Shade A Noviol) was interposed in the light beam traversing the wedge of the photometer. Although this filter did not entirely eliminate the hue difference, no trouble was experienced in making readings, surprisingly close duplicates were obtained, and it was found possible to determine lactic acid in quantities as small as 10 p.p.m.

The readings obtained on the photometer, necessarily, must be applied to a standard curve in order to determine the lactic acid present in the sample. Such a curve can be prepared by allowing up to 12 mg. of lactic acid (as lithium lactate) to go through the carbon treatment as directed in the method. Fig. 2 is an example of such a curve. Lithium lactate was chosen as the source of lactic acid because it is anhydrous and non-hygroscopic, advantages that zinc lactate does not possess. The procedure for preparing lithium lactate is given in the method.

A photometer reading representing 12 mg. of lactic acid is the upper limit to which the curve was carried, because higher than this, with the filter employed, the solutions appear too dark in the photometer for accurate readings. Inasmuch as the curve does not follow Beer's law, an equation can not conveniently be used to compute lactic acid from the photometer readings. It will be necessary for each analyst to construct his own standard curve.

Experiments showed that exposure to daylight causes fading of the yellow color amounting to 25 per cent in one hour, and 40 per cent in two hours. Therefore the prepared color solution was placed in a Nessler tube wrapped in black paper. By this means the solution did not show any perceptible fading after 48 hours of standing.

The method follows:

COLORIMETRIC DETERMINATION OF LACTIC ACID IN MILK AND MILK PRODUCTS

REAGENTS

(a) *Lithium lactate.*—Prepare the salt from sirupy lactic acid and lithium carbonate. To one volume of lactic acid add 2 volumes of water, heat to boiling, and add lithium carbonate in small portions until the solution is neutral or slightly alkaline to phenol red indicator. (A dilute lactic acid solution is added to slight acidity in order to obviate the presence of lithium carbonate during crystallization.) Evaporate until lithium lactate starts to crystallize. Add five volumes of alcohol and allow the mixture to stand overnight. Filter on a Büchner funnel and wash several times with alcohol. Purify the lithium lactate by crystallization from water and dry at 100°C. 106.6 mg. of lithium lactate = 100 mg. of lactic acid.

Carbon.—To 10 grams of a high-grade carbon in a 600 cc. beaker, add about 200 cc. of water and 30 cc. of normal HCl and place on the steam bath for 20 minutes. Agitate continuously with air. Filter on a Büchner funnel and suck as dry as possible, tamping with a flattened stirring rod. Transfer the cake to a beaker, add about 200 cc. of water, mix thoroughly, and refilter. Repeat the washing and filtering twice, and dry in the water oven.

Ferric chloride solution.— Dissolve 2 grams of $FeCl_3$, analytical reagent, in water; add 5 cc. of normal HCl and dilute to 200 cc.

PROCEDURE

To 50 grams of fluid milk, or 5 grams of a dried milk product thoroughly mixed with about 50 cc. of water (in case of cream and ice cream use 20 grams of material made to about 50 cc. with water), add with constant stirring 6 cc. of normal H_2SO_4, followed by 5 cc. of a 20% phospho-tungstic acid solution (1 cc. in case of cream, 2 cc. in case of ice cream). Transfer the mixture with water to a 100 cc. volumetric flask, breaking any foam with a few drops of alcohol and a stirring rod. Make to mark, shake, allow to stand several minutes, and filter through a folded paper. Extract the solution in the continuous extractor with about 200 cc. of washed ether. To insure uniform boiling, suspend a piece of thread in the ether. Into the inner tube of the extractor pipet 50 cc. of the solution. Add 0.5 cc. of H_2SO_4 (1+1) and extract for 2 hours. (An electric hot plate is the most practical means of heating the ether, and to prevent superheating the extraction flask may be placed on the previously heated plate. The condensing ether should return to the flask in a steady stream. The outlet of the condenser should not be less than ½″ inside diameter, otherwise the condensing ether may regurgitate.)

To the flask containing the ether extract add 20 cc. of water and expel the ether on the steam bath. Neutralize the contents of the flask with saturated barium hydroxide solution (phenolphthalein indicator). Transfer to a 110 cc. volumetric flask with alcohol until the volume is about 90 cc. Heat almost to boiling on the steam bath, cool, make to mark with alcohol, and filter through a quantitative paper. Evaporate 100 cc. of the filtrate over an open flame to about 10 cc., add about 50 cc. of H_2O, and again evaporate to about 10 cc. Add from a buret 3.3 cc. of 0.1 *N* HCl and transfer the contents of the beaker with water to a 55 cc. volumetric flask until the volume is about 40 cc. Add 200 mg. of the acid-treated carbon, immediately shake, and place on the steam bath for 10 minutes, mixing at frequent intervals. Cool, make to mark with water, and filter through a quantitative paper, pouring back until bright.

Make a preliminary estimation of the quantity of lactic acid present in the following manner: Transfer 10 cc. of the filtrate to a Nessler tube. (A total of 3 cc. of 0.1 *N* HCl is required in the tube. Each 10 cc. of the filtrate contains 0.6 cc. of 0.1 *N* HCl added during the carbon treatment.) To provide the 3 cc. of 0.1 *N* HCl required, add 2.4 cc. of the acid and make to about 40 cc. with water. Place the tube in a jacket of black paper, add from a buret 5 cc. of the $FeCl_3$ solution, make to the 50 cc. mark, and mix. (After the color has been developed, dilution for the purpose of reducing the color intensity is not permissible.) Fill a 4″ cell, the walls of which are painted black, with the solution and read in the photometer. Estimate the quantity of lactic acid present in the 10 cc. portion taken from the standard curve of the instrument. (The approximate percentage of lactic acid present having thus been ascertained, the quantity of solution that will not overstep the 12 mg. limit may be easily calculated.)

If a photometer is not available, make the final estimation of lactic acid in Nessler tubes. Prepare a solution of lithium lactate, 1 cc. of which is equivalent to 1 mg. of lactic acid (106.6 mg. of lithium lactate made to 100 cc. with water). Make a preliminary estimation of the quantity of lactic acid present in the determination in the following manner: Transfer 5 cc. of the lithium lactate solution to a Nessler tube. Add 3 cc. of 0.1 *N* HCl, make to about 40 cc. with water, wrap the tube in black paper, add 5 cc. of the $FeCl_3$ solution, dilute to the 50 cc. mark, and mix. Compare with the color produced by the 10 cc. portion of the filtrate as prepared above. If the two samples do not match, repeat, using a quantity of the lithium lactate solution that will produce an approximate match. Having thus ascertained the approximate quantity of lactic acid present, transfer a portion of the remaining filtrate that will contain not more than 10 mg. of lactic acid to a Nessler tube and produce the color with $FeCl_3$ as directed previously. Knowing

approximately the quantity of lactic acid present in the portion taken, prepare standards from the lithium lactate solution, allowing the standards to go through the carbon treatment as directed in the method.

For computing the lactic acid it is necessary to know the quantity of insoluble solids in the portion taken for analysis. The data are contained in Table 2.

TABLE 1.—*The average protein and fat content of milk products*
(Sp. gr. of butterfat, 0.9; of casein, 1.3)

PRODUCT	FAT	PROTEIN
	per cent	*per cent*
Whole Milk	3.62†	3.55†
Dry Whole Milk	26.0†	27.2†
Evaporated Whole Milk	8.63*	7.27*
Skim Milk	0.15†	3.72†
Dry Skim Milk	1.00†	37.4†
Sweetened Condensed Milk	9.28*	8.36*
Dry Buttermilk	5.87†	38.74†

† Fundamentals of Dairy Science, by Associates of Rogers, 2nd Ed.
* Hunziker, Condensed Milk and Milk Powder, 4th Ed. (1926).

TABLE 2.—*Recovery of lactic acid added to dry skim milk*
(Lactic acid in 5 g. of milk used, 0.0012 g.)

LACTIC ACID ADDED	LACTIC ACID RECOVERED	DIFFERENCE
grams	*grams*	*gram*
	0.0015	+0.0005
0.001	0.0012	+0.0002
	0.0052	+0.0002
0.005	0.0052	+0.0002
	0.0150	0.0000
0.015	0.0149	−0.0001
	0.0245	−0.0005
0.025	0.0248	−0.0002
	0.0388	−0.0012
0.040	0.0391	−0.0009
	0.0487	−0.0013
0.050	0.0492	−0.0008
	0.0745	−0.0005
0.075	0.0743	−0.0007

For example, in the case of whole milk the 50 grams will contain 3.4 cc. of insoluble solids—$\frac{50}{100}\left(\frac{3.62}{.9}+\frac{3.55}{1.3}\right)=3.4$. With the figure thus obtained, the quantity of material in the final aliquot is computed as follows:

$$\frac{W}{100\text{-}S}\times\frac{50}{110}\times\frac{100}{55}\times A, \text{ in which}$$

S = cc. of insoluble solids;
A = cc. of aliquot taken in the final analysis; and
W = weight of sample.

In order to test the accuracy of the method, it was tried out on a spray dried skim milk of good quality. The milk showed a lactic acid content on the reconstituted basis of 0.0021 gram per 100 grams. Varying quantities of lactic acid as lithium lactate were added to 5 gram portions of this milk. The recoveries obtained by the proposed method are shown in Table 2, where it will be seen that closely agreeing duplicates were obtained, and that the method recovers practically 100 per cent of the lactic acid added.

The method was applied to various milk products. The results obtained are given in Table 3.

TABLE 3.—*Lactic acid content of milk products determined by the proposed method*

PRODUCT	LACTIC ACID	LACTIC ACID ON BASIS OF RECONSTITUTED WHOLE MILK	PRODUCT	LACTIC ACID	LACTIC ACID ON BASIS OF RECONSTITUTED WHOLE MILK
	g./100 g.	*g./100 g.*		*g./100 g.*	*g./100 g.*
Whole Milk	0.0021		Sweetened Condensed Milk	0.0012	
Wagon Delivery	0.0022	0.002	Commercial	0.0009	—
Whole Milk	0.0042		Dried Skim Milk	0.533	
Wagon Delivery	0.0039	0.004	Commercial	0.522	0.048
Whole Milk	0.0029		Dried Skim Milk	0.442	
Wagon Delivery	0.0028	0.003	Commercial	0.426	0.039
Whole Milk	0.0021		Dried Skim Milk	0.460	
Wagon Delivery	0.0022	0.002	Commercial	0.451	0.041
Whole Milk	0.0056		Dried Skim Milk	0.689	
Wagon Delivery	0.0055	0.006	Commercial	0.710	0.064
	0.0012		Dried Skim Milk	0.098	
	0.0015	0.001	Commercial	0.095	0.009
Evaporated Milk	0.0062		Dried Skim Milk	0.424	
Commercial	0.0062	0.003	Commercial	0.408	0.038
Evaporated Milk	0.0142		Dried Skim Milk	0.975	
Commercial	0.0147	0.007	Commercial	0.997	0.089
Evaporated Milk	0 0076		Dried Buttermilk	0 851	
Commercial	0.0076	0.004	Commercial	0.850	—
Dried Whole Milk	0.0396		Dried Buttermilk	0.850	
Commercial	0.0370	0.005	Commercial	0.866	—

TABLE 3.—(*Continued*)

PRODUCT	LACTIC ACID	LACTIC ACID ON BASIS OF RECONSTITUTED WHOLE MILK	PRODUCT	LACTIC ACID	LACTIC ACID ON BASIS OF RECONSTITUTED WHOLE MILK
	g./100 g.	*g./100 g.*		*g./100 g.*	*g./100 g.*
Dried Whole Milk	0.0218		Malted Milk	0.177	
Commercial	0.0226	0.003	Commercial	0.186	—
Dried Skim Milk	0.0238		Liquid Chocolate	0.009	
Authentic	0.0238	0.002	Milk Wagon Delivery	0.009	—
Dried Skim Milk	0.0328		Evaporated Milk	0.043	
Authentic	0.0358	0.003	Commercial	0.043	*0.022
Dried Skim Milk	0.0734		Cream, Authentic	0.0013	
Authentic	0.0686	0.006	20 per cent	0.0015	—
Dried Skim Milk	0.0570		Cream, Authentic	0.0007	
Authentic	0.0570	0.005	30 per cent	0.0010	—
Dried Skim Milk	0.0458		Ice Cream Vanilla	0.0130	
Commercial	0.0446	0.004	Commercial	0.0135	—
			Ice Cream Vanilla	0.0150	
			Maple Nut		—
			Commercial	0.0150	

BUTTER

The general method for the determination of lactic acid in butter is the same as that described previously. However, it is necessary to remove most of the fat. To do this, proceed as follows:

Weigh 20 grams of the material into a 100 cc. beaker. Add 15 cc. of water and 0.5 cc. of normal H_2SO_4 and place on the steam bath for 10 minutes, stirring frequently. Neutralize with normal NaOH and transfer the contents of the beaker to an 8 oz. centrifuge bottle with 15 cc. of water. Add 50 cc. of ether and mix well, avoiding violent shaking. Add 50 cc. of petroleum ether, mix well, and centrifuge. Draw off the ether layer as completely as possible by means of a syphon having its lower end bent upwards. Repeat the extraction, using 25 cc. of each of the ethers. Transfer the residue in the bottle with water to a 100 cc. volumetric flask, add 3 cc. of normal H_2SO_4, and place the flask in hot water to expel the ether. (Care should be taken not to agitate the material in the flask too vigorously, otherwise some loss may result through violent evolution of the ether.) Cool the mixture, and precipitate the proteins with the phosphotungstic acid solution, adding the reagent dropwise, until no further precipitation occurs. Make to mark, shake, and filter. Transfer 50 cc. of the filtrate to the continuous extractor and proceed with the determination as directed for milk and milk products.

Lactic acid was determined on good quality sweet cream butter. It showed an acid content of 0.0016 gram per 100 grams. Varying quantities

of lithium lactate were added to 20 gram portions of this butter. The recoveries are shown in Table 4.

TABLE 4.—*Recovery of lactic acid added to butter*

LACTIC ACID ADDED	LACTIC ACID RECOVERED	DIFFERENCE
gram	*gram*	*gram*
	0.0010	0.0000
0.001	0.0010	0.0000
	0.0048	−0.0002
0.005	0.0049	−0.0001
	0.0147	−0 0003
0.015	0.0144	−0.0006
	0.0241	−0.0009
0.025	0.0244	−0.0006

TABLE 5.—*The lactic acid content of butter*

SAMPLE	LACTIC ACID	ACIDITY OF THE CREAM AS LACTIC AT TIME OF CHURNING
	g./100 g.	*g./100 g.*
	0.0016	
1	0.0016	0.15
	0 0066	
2	0 0065	0.20
	0 0087	
3	0.0087	0.30
	0.0344	
4	0 0344	0.40
	0 0202	
5	0 0193	0.25*
	0.0381	
6	0.0375	0.20†
Sweet cream butter	0.0044	
Market Sample	0.0039	
	0.0557	
Market Sample	0.0557	

* Original acidity of cream 0.4%, reduced with magnesia lime to 0.25%.
† Original acidity of cream 0.58%, reduced with magnesia lime to 0.20%.

W. White of the Bureau of Dairying of the Department of Agriculture kindly furnished the writer with some authentic samples. These butters (all cream pasteurized) were made in April, 1936, and stored at 0°F. Lactic acid was determined on these butters, on a commercial butter, and on a commercial sweet cream butter. Results are shown in Table 5.

SUMMARY

A method applicable to milk and milk products generally is proposed for the colorimetric determination of lactic acid. The various steps in the procedure are not difficult, and the determination can be easily completed in a working day. The determination of lactic acid in as minute quantity as 10 p.p.m. is possible.

RESIN AND VOLATILE OIL IN CUBEBS

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N. Y.)

Cubebs are usually imported from Singapore and are grown almost entirely in the East Indies.

During the past five years many of the importations of cubebs in New York have been analyzed for the yield of resins and volatile oil. Determinations have also been made of some of the physical and chemical characteristics of these oils. Approximately 10 per cent of these importations were found to contain spurious fruits, in some instances as much as 50 per cent. The results reported here were obtained by the method outlined in *Methods of Analysis, A.O.A.C.*, 1935, 450, which has been adopted as tentative for the evaluation of ginger.

AUTHENTIC CUBEBS

RESIN	YIELD v/w*	SP. GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	AC. NO.	EST. NO.
per cent						
8.3	17.0	0.913	−19.7	1.498	0.4	4.9
7.78	14.0	0.919	−26.3	1.497	0.5	4.3
7.0	15.0	0.917	−24.2	1.497	0.8	1.0
8.2	20.0	0.911	−27.5	1.495	0.7	10.4
6.44	13.6	0.917	−29.4	1.495	0.5	3.0
8.32	13.1	0.913	−36.6	1.495	0.6	5.0
6.54	12.5	0.913	−26.3	1.496	1.0	10.7
7.88	12.9	0.917	−39.1	1.494	0.87	5.4
8.30	15.3	0.917	−36.0	1.498	0.35	3.0
8.47	16.4	0.919	−46.0	1.493	0.67	4.2
8.32	16.4	0.918	−38.7	1.492	0.80	3.6

ADULTERATED CUBEBS

ADULTERATED	RESIN	YIELD v/w*	SP. GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	AC. NO.	EST. NO.
per cent	*per cent*						
50	7.71	17.0	0.879	+22.5	1.476	0.5	5.4
12	6.58	14.2	0.910	−17.3	1.492	1.8	4.5
50	6.6	10.3	0.905	+ 2.4	1.491	0.5	3.6

* cc. per 100 grams of cubebs.

CONCLUSIONS

1. The yield of resins and volatile oil in authentic cubebs was found to be within the range ordinarily expected.
2. The physical and chemical characteristics of the oils of the authentic cubebs were also found to be fairly uniform.
3. Adulteration of cubebs is indicated by the physical characteristics of the volatile oil obtained.

BOOK REVIEWS

Soil Science—Its Principles and Practice. By W. W. WEIR. 615 pages, 134 illustrations. J. B. Lippincott Company, Chicago and Philadelphia, 1936.

This text of 27 chapters is by an author of both academic and commercial background. The subject matter is arranged according to the classification adopted by The International Society of Soil Science. Clarity and simplicity are achieved in the presentations, mastery of which will leave one well grounded in the Science of Pedology, of which the author desires to present "a bird's-eye view." The book has a good make-up and is well illustrated and charted.

The Author shows the results of a comprehensive compilation and evidences a grasp of most of the aspects embraced in the text. In a treatise of such scope as that comprised in the 27 divisions of subject matter, it is to be expected that the topics treated would be handled with variant emphasis and extent, as a reflection of the Author's particular interest and degree of familiarity with the several phases of Soil Science.

One might be privileged to differ with the Author as to the importance of certain inclusions and the exclusion of available data of pertinent character. One finds a statement that certain information is not available, although informative data have been published by bulletins and journal articles from two American Experiment Stations. One may also find a correct statement without supporting citations. A Soil Chemist is struck with the omission of any reference to either of the two publications, Wiley's "Principles and Practice" and Frear's "Sour Soils and Liming."

In general, the Author has achieved his intent to produce a useful text and to point the student toward authoritative sources of information. The volume should prove an acceptable addition to student, teacher, and devotee of Soil Science.—W. H. MACINTIRE.

Handbook of Chemistry and Physics. CHARLES A. HODGMAN, Editor in Chief. 21st Edition, August, 1936. Published by the Chemical Rubber Publishing Co. Cleveland, Ohio. Price $6.00.

This annual revision of this useful handbook, according to the preface, while only increased by 64 pages includes 175 pages of new composition. A table of haversines has been added. The sections on laboratory and photographic recipes have been enlarged. Pace is kept with new development in science and industry by the addition of six pages of statistical formulae and tables and 17 pages on the properties of commercial plastics.—H. A. LEPPER.

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Two communications were received from the American Society of Brewing Chemists signed by Dr. Robert Schwarz, Chairman. One of these designates Mr. Philip Gray as representative of the American Society of Brewing Chemists and Dr. Stephen Laufer as alternate.

Correspondence with some of the newer members of the Alcoholic Beverages Section has led to the belief that some of the chemists—some of the newer members—do not have a clear understanding of the purposes of this Association. Therefore it might not be out of place to call the attention of these newer members to a statement in the program and to certain provisions in our constitution. The statement in the program is as follows: "The A.O.A.C. is unique in that no membership dues are assessed; the eligibility of every member is conceded by virtue of the position he occupies, and it continues only so long as such position is held." In the constitution the objects of the Association are stated as follows: "To secure, devise, test and adopt uniform and accurate methods for the analysis of fertilizers, soils, foods, feeding stuffs, dairy products, insecticides and fungicides and other materials relating to agricultural pursuits; also medicinal products, caustic poisons; paints, paint materials, and varnishes; second, to secure uniformity in the statement of analytical results." Among the duties of the referees are the following: "To direct and conduct research on the methods and subjects assigned to them; to prepare and distribute samples and standard reagents to the members of the Association and others; to present at annual meetings the results of their work and recommendations for methods to be based thereon, and to direct and encourage general discussion." The papers presented at the meeting are reviewed by a subcommittee to determine their suitability for publication in the *Journal*.

REPORT ON BEER, MALT, AND MALT ADJUNCTS

By J. A. LE CLERC (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In this study collaboration was obtained from chemists connected with the following institutions and industries: Schwarz Laboratories, E. A. Siebel and Company, Siebel Institute of Technology, Wahl-Henius Institute, Wallerstein Laboratories, Anheuser-Busch Incorporated, Premier-Pabst Corporation, Jos. Schlitz Brewing Company, Corn Products Refining Company, the Patent Cereals Company, Albert Schwill and

TABLE 1.—*Beer*

COLLABORATORS	(1)	(2)	(3)	(8)	(7)	(6)	(4)	(12)	(14)
Color	6.5	6.5	6.0	6.0	7.0	7.0	6.7	—	7.5
Specific Gravity	1.01736	1.01708	1.01705	1.01718	1.01691	1.01644	1.01708	1.01733	1.01705
Apparent Extract (%)	4.43	4.4	4.35	4.37	4.31	4.18	4.35	4.42	4.35
Alcohol by Volume (%)	4.71	—	4.49	4.55	4.64	4.70	4.70	4.57	4.64
Alcohol by Weight (%)	3.66	3.70	3.57	3.62	3.60	3.65	3.68	3.62	3.63
Alcohol by Imm. Refract. (%)	—	—	3.67*	—	—	—	—	4.63†	—
Real Extract (%)6	6.27	—	5.97	6.00	5.98	5.88	—	6.07	6.14
Real Extract (%)7a	6.09	6.00	6.04	—	—	—	—	6.08	6.08
Real Extract (%)7(b)	—	—	6.00	—	—	—	6.14	6.27	6.07
Real Extract (%)7(c)	—	6.04	—	—	—	—	6.05	6.02	6.03
Extract Original Wort (%)	13.31	13.12	12.86‡	13.14	12.92	12.90	13.21	—	13.08
Real Degree Fermentation (%)	52.90	54.26	53.58‡	54.20	53.72	54.52	53.80	—	53.90
Total Acid (%)	0.175	0.191	0.19	0.20	0.23	0.20	0.19	0.216	0.219
Reducing Sugars (%)	1.82	—	—	—	1.81	1.56	1.78	1.80	1.81
Dextrin (%)	2.81	2.63	2.67	—	2.30	2.60	2.05	2.79	2.86
Direct Polarization (°)	43.2	—	42.5	42.2	—	—	38.3	42.5	42.1
Ash (%)	—	—	—	—	—	0.202	—	—	—
Phosphoric Acid (%)	—	—	—	—	—	0.07	—	—	—
Protein (%)	—	—	—	0.60	—	0.56	—	—	—
CO_2 (%)	—	0.38	0.47	—	0.44	—	0.46	—	—
SO_2 (mg. per liter)	3.84	4.05	13.0	2.9	7.6	8.78	12.0	7.00	2.6
I_2-Reaction, 22(a)	complete	complete	complete	complete	complete	—	no change	complete	complete
I_2-Reaction, 22(b)	normal	—	—	—	—	—	—	—	—
Coloring Matter	—	—	none	—	—	—	—	—	—
Metals (As.) p.p.m.	—	—	0.016	—	none	—	—	—	—
Metals (Cu.) p.p.m.	—	—	1.760	—	none	—	—	—	—
Pasteurization	yes	no	—	yes	—	—	yes	no	no
Chlorides (%)	0.017	0.00071	0.0033§	—	—	0.26	0.013	0.0007§	0.149
Methyl Alcohol (%)	—	—	—	—	—	—	—	—	Absent
*p*H	—	—	—	4.7	4.90	4.66	—	—	—
App. Deg. Attenuation	—	—	—	66.60	—	67.62	—	—	—
Fe (p.p.m.)	—	—	—	—	0.50	—	—	—	—

* By weight.
† By volume weight.
‡ Based upon 5.97 Extract.
§ As NaCl.

Company, North Dakota Regulatory Department, The Bureau of Agricultural Economics, and The Food and Drug Administration. Of these 9 assisted in the study of beer methods, 10 of malt methods and 11 in the study of methods for the analysis of malt adjuncts.

BEER

Every method for the analysis of beer, whether official or tentative (with the exception of the methods for volatile acid and glycerol) was tested by one or more collaborators. The results of these tests are given in Table 1. They are summarized as follows:

Sec. 2, Color, *Tentative*.—The results of eight collaborators vary from 6.0 to 7.5.

Sec. 4, Apparent Extract, *Tentative*.—The results from nine collaborators vary from 4.18 to 4.42 per cent. Eight of the collaborators obtained results varying from 4.31 to 4.42 per cent.

Sec. 5(a), Alcohol by Volume, *Official*.—Eight collaborators studied this method. The results vary from 4.49 to 4.71 per cent. One of the collaborators recommends that 100 grams of beer be taken instead of 100 cc. and that the distillate be made up to 100 grams instead of 100 cc.

Sec. 5(b), Alcohol by Weight, *Official*.—The results of nine collaborators varied from 3.57 to 3.70 per cent. One collaborator states that the corresponding section, 25(c), p. 137 of the 3rd ed., should supersede section 5(b) of the 4th ed., because the latter is incorrect.

Sec. 6, Real Extract, *Official*.—Seven collaborators obtained results, minimum, 5.89; maximum, 6.27 per cent.

Sec. 7(a), Real Extract, *Tentative*.—The results of five collaborators agree very closely (6.00–6.09 per cent).

Sec. 7(b), Real Extract by Immersion Refractometer, *Tentative*.—The results of five collaborators agree fairly closely with those obtained by the same collaborators using 7(b) or 7(c).

Sec. 7(c), Real Extract, *Tentative*.—The results obtained by four collaborators show a close agreement with results obtained by methods 7(a) or 7(b).

Sec. 8, Extract of Original Wort, *Tentative*.—Seven chemists obtained results varying from 12.86 to 13.31 per cent. One collaborator states that by using the formula given in the third edition of *Methods of Analysis* the extract was 13.19, whereas the new formula gives 12.90 per cent. Another collaborator states that "E," in the new formula, should be derived from Sec. 7(c) instead of from Sec. 6.

Sec. 9, Real Degree of Fermentation, *Tentative*.—This is dependent upon Sec. 8. The variation in the results was 52.90–54.52 per cent.

Sec. 10, Total Acid, *Tentative*.—The variation in the results of nine collaborators is from 0.175 to 0.23 per cent. One collaborator, whose results are below the average, states that the color interferes with the sharpness of the end point and hence results are likely to be too high. Another collaborator recommends omitting the words "to remove CO_2." A third prefers using a steam bath and to hold the beer at this temperature for a definite length of time.

Sec. 12, Reducing Sugars, *Official*.—Fairly satisfactory results were obtained by six collaborators (1.56–1.82 per cent). One chemist suggests a modification as follows:

To 50 cc. of beer, in a 100 cc. flask, add 10 cc. of Pb-acetate, make up to volume, shake, and filter. Proceed as directed in the Munson-Walker method without "de-leading."

Sec. **13**, Dextrin, *Tentative*.—Eight collaborators obtained results varying from 2.05 to 2.86 per cent.

Sec. **14**, Direct Polarization, *Tentative*.—Five collaborators obtained very closely agreeing results (42.1–43.2°), but the results of the sixth collaborator deviated considerably (38.8°). One collaborator suggests that Pb-acetate be used as a clarifying agent and that the lead be removed before polarization.

Sec. **19**, CO_2, *Tentative*.—Only four collaborators took part in the study of this method. The results vary from 0.38 to 0.47 per cent. The comments state (a) that the CO_2 is not removed completely, (b) that the directions are not clear as to the type of "distilling head," (c) that a statement should be made that the H_2SO_4 must be added slowly up to the phenolphthalin end-point, and (d) that the method of Cannissaro (*Ind. Eng. Chem.*, 1923, p. 1074) and that found in *J. Inst. Brew.*, 1929, p. 480 are simpler.

Sec. **21**, SO_2, *Tentative*.—Nine collaborators took part in this study. The results of the collaborators vary widely, the minimum amount found being 2.6 p. p. m., the maximum 13. The collaborators state (a) that beer should not be decarbonated, (b) that bromine gives too high results, (c) that H_2O_2 should be used instead of Br or I and distillation be conducted in a stream of CO_2, and (d) that straight distillation should replace "distillation with steam."

Sec. **22(a)**, Iodine Reaction, *Tentative*.—There was complete agreement among the eight collaborators.

Sec. **26**, Pasteurization, *Tentative*.—Three of the collaborators found a marked difference in sugar content of the two samples, indicating that the beer had not been pasteurized; the results of three other collaborators indicated the reverse. It was suggested that clarification with Pb-acetate be omitted and that the test was unsatisfactory.

Sec. **27**, Chlorides, *Tentative*.—Here again wide variations in results were obtained by the seven collaborators, the minimum being 0.0007 per cent (obtained by two chemists) and the maximum 0.26 per cent. One comment was that this method is no improvement over the method by which the NaCl is determined in the ash.

RECOMMENDATIONS[1]

It is recommended—

(1) That the following methods be advanced to the status of "official, first action:" Sec. **2**, Color; Sec. **4**, Apparent Extract; Sec. **7 (a)**, **(b)** and **(c)**, Real Extract; and Sec. **22 (a)**, Iodine Reaction; and that Sec. **5 (b)** be rewritten.

(2) That the following methods be further studied: Sec. **8**, Extract of Original Wort; Sec. **9**, Real Degree of Fermentation; Sec. **10**, Total Acid; Sec. **12**, Reducing Sugars; Sec. **13**, Dextrin; Sec. **14**, Direct Polarization; Sec. **19**, CO_2 (compare this method with other methods, *e. g.*, Cannissaro's); Sec. **21**, SO_2; Sec. **26**, Pasteurization; and Sec. **27**, Chlorides.

(3) That studies be also made of methods for the determination of heavy metals, especially Fe, Cu, Pb, as well as As and F; preservatives; and *p*H.

MALT

The methods used were those already adopted by the American Society of Brewing Chemists. The results are given in Table 2.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 63 (1937).

TABLE 2.—*Malt and grits*

COLLABORATORS	(3)	(5)	(1)	(4)	(11)	(6)	(7)	(2)	(8)	(13)
Bushel Weight (lb.)	39.1	38.0	—	37.5	39.0	39.2	37.2	39.2	38.2	—
1000 Kernel Weight, as is (grams)	24.4	26.5	24.4	23.9	23.9	24.8	23.8	24.6	23.5	24.4
1000 Kernel Weight, dry (grams)	22 7	24.8	22.6	22.2	22.2	23.0	22.2	22.9	21.9	22.6
Growth, 0–¼ (%)	6.0	3.0	9.0	2.0	3.0	4.0	2.0	3.0	—	1.0
¼–½ (%)	4.0	4.0	4.0	3.0	2.0	1.0	2.0	5.0	—	4.0
½–¾ (%)	7.0	5.0	15.0	2.0	4.0	1.0	10.0	21.0	12.0	20.0
¾–1 (%)	78.0	88.0	71.0	92.0	91.0	93.0	86.0	68.0	84.0	62.0
Overgrown (%)	5.0	—	1.0	1.0	—	1.0	—	3.0	4.0	1.0
Assort. on 8/64″ screen (%)	2.7	—	0.6	5.2	5.4	3.5	3.5	3.4	0.6	—
7/64″ screen (%)	13.0	10.9	4.8	8.9	8.0	14.9	14.2	13.4	9.2	—
6½/64″ screen (%)	48.0	61.1	21.7	53.6	49.4	52.7	50.6	59.4	34.2	—
5½/64″ screen (%)	29.9	22.4	59.7	27.8	31.6	24.8	26.7	18.8	47.5	—
5/64″ screen (%)	3.9	4.2	9.7	3.2	4.9	3.4	4.4	3.8	5.2	—
through 5/64 screen (%)	1.5	1.4	3.8	1.3	0.7	0.7	0.6	1.2	3.3	—
Foreign Seeds (%)	0.9	1.0	—	1.1	0.6	0.6	0.5	0.6	neg.	—
Broken Kernels (%)	0.1	0.4	0.4	0.5	0.4	0.3	0.3	1.1	—	—
Mold	trace	none	none	none	none	1.6	none	none	none	—
Mealiness-mealy (%)	90.0	88.0	91.0	81.0	91.0	96.0	95.0	95.0	91.0	—
½ glassy (%)	9.0	8.0	3.0	11.0	7.0	2.0	4.0	4.0	8.0	—
glassy (%)	1.0	4.0	6.0	8.0	2.0	2.0	1.0	1.0	1.0	—
Moisture (%)	6.8	6.7	7.5	7.10	6.9	7.2	6.9	7.1	6.9	6.9
Extract (fine), as is, (%)	68.6	67.2	67.4	67.6	67.8	67.7	67.5	68.9	67.6	67.8
Extract (fine), dry (%)	73.6	71.9	72.9	72.8	72.9	73.0	72.5	74.2	72.6	72.8
Extract (coarse), as is (%)	67.2	66.3	67.1	66.9	66.9	—	—	—	—	—
Extract (coarse), dry (%)	72.1	71.0	72.5	72.0	71.9	—	—	—	—	—
Color of wort	1.5	1.8	1.9	1.7	1.7	1.9	1.8	2.2	1.7	1.6
Odor of mash	sl. arom.	arom.	arom.	—	—	sl. arom.	arom.	arom.	sl. arom.	sl. arom.
Degree of clarity	clear	clear	clear	—	clear	clear	clear	clear	clear	clear
Speed of filtration	normal	normal	normal	—	normal	normal	normal	normal	normal	normal
Conversion (min.)	5–7	−5.0	−5.0	−5.0	−5.0	−5.0	5–7	5.0	−5.0	−10.0
D. P. Lintner (dry)	93.8	102.0	102.0	113.0	105.0	106.0	106.0	114.0	112.0	105.0

The results of collaboration on these methods, all of which are tentative, follow:

Sec. **31**, Bushel Weight.—The lowest of eight tests was 37.2 pounds, the highest 39.2.

Sec. **32**, Length of Acrospire.—Ten chemists collaborated. The results obtained vary as follows: 0–¼, 0–9 per cent; ¼–½, 0–5 per cent; ½–¾, 1–21 per cent; ¾–1, 62–93 per cent; overgrown, 0–5 per cent.

Sec. **33**, Mealiness.—The results of nine collaborators show the following: mealy, 81–96 per cent; ½ glassy, 2–11 per cent; glassy, 1–6 per cent.

Sec. **34**, 1000 Kernel Weight.—The lowest figure obtained was 23.5 grams, the highest 26.5 grams. Ten chemists collaborated.

Sec. **35**, Assortment.—Nine chemists collaborated and obtained the following results:

Screen	*per cent*
On 8/64″..............	0– 5.4
On 7/64″..............	4.8–14.9
On 6½ /64″............	21.7–61.1
On 5½ /64″............	18.8–59.7
On 5/64″..............	3.2– 9.7
Through 5/64″.........	0.6– 3.8

TABLE 3.—*Grits*

COLLABORATORS	(3)*	(5)	(1)	(10)	(4)	(9)	(11)	(6)	(7)	(2)	(8)
Moisture (%)........	11.2	11.7	11.3	11.80	11.50	10.92	11.7	11.9	10.8	12.1	11.0
Extract, as is (%)....	81.6	75.0	80.8	76.5	80.16	74.6	80.3	78.3	78.0	78.8	81.1
Extract, dry (%).. ..	91.9	85.0	91.1	86.75	90.60	83.9	90.9	88.9	87.4	89.6	91.1
Fat, as is (%)..	0.78	0.90	0.83	0.63	0.77	0.90	0.77	0.76	0.82	0.59	0.93
Fat, dry (%)........	0.89										
Ash, as is (%).......	0.40										
Ash, dry (%)........	0.45										
Conversion time (min.)			9.0	10.0	7–10			12			
Filtration...........								Clear	Normal		Normal

* Collaborator 3 reported the following sieve tests: 20-mesh, 81.2%; 40-mesh, 16.8%; 60-mesh, 0.5%; 80-mesh, 0.3%; 100-mesh, 0.2%; through 100-mesh, 1.0%.

Secs. **36** and **37**, Mold, Foreign Seeds and Broken Kernels.—The agreement among the nine collaborators was very close.

Secs. **38–40**, Moisture.—Ten collaborators took part in this test. The results ranged from 6.7 to 7.2 per cent.

Sec. **44**, Extract (fine).—Ten collaborators participated. Results: Dry basis, 71.9–74.2 per cent. Extract (coarse).—Five collaborators participated. Results: Dry basis, 71.0–72.5. Comment regarding coarse grist: Not over 30 per cent should pass through the No. 30 sieve.

Sec. **45**, Color of Wort, Using ½″ Cell.—Ten collaborators participated. Results ranged from 1.5 to 2.2. All collaborators were agreed that the odor of the wort was slightly aromatic, that the speed of filtration was normal, the degree of clarity "clear," and the time of conversion less than 10 minutes.

Sec. **46**, Diastatic Power.—Ten collaborators participated. Results: Minimum, 93.8; maximum, 114 per cent.

It was suggested that results should always be reported on both "dry" basis and "as is" basis, and that a glass counterpoise having a displacement within 5 cc. of that of the pycnometer should be used when specific gravity is determined.

RECOMMENDATIONS

It is recommended—

(1) That the study of the methods for bushel weight, length of acrospire, mealiness, 1000 kernel weight, assortment, and diastatic power be repeated.

(2) That the following methods be made official, first action, **41**, Extract—*fine grinding* and *coarse grinding;* **38**, Moisture; and **45**, Color of Wort.

MALT ADJUNCTS

The results and comments of the collaborators follow:

Sec. **49**, Moisture.—The results were minimum, 10.8; maximum, 12.1 per cent. Heating at 100° in vacuum was reported as preferable to a temperature of 103–104° at ordinary pressure, and it was suggested that the grits be ground to a definite degree of fineness.

Sec. **50**, Fat.—Results were minimum, 0.59; maximum, 0.93 per cent. It was suggested that the temperature of drying be stated.

Sec. **51**, Extract "dry basis."—Results were minimum, 83.9; maximum, 91.9 per cent. It was suggested (a) that "finely" ground malt instead of "crushed" be used, (b) that the malt should be coarse, (c) that the sample should not be boiled and a steam bath should be used, (d) that a portion of the malt be added to the grits in the boiling operations, and (e) that an iodine test of the grits after mashing be added to the methods.

RECOMMENDATIONS[1]

It is recommended—

(1) That the vacuum oven method (**XX**, Sec. **2**) for moisture be compared with the method given in **XIV**, **53**.

(2) That a special study be made of methods for fat that might be applicable to corn grits, broken rice, and flakes.

(3) That a study of the method for extract be made, and consideration given to the suggestion to use a portion of the malt in the boiling operations.

The thanks of the referee and of this section should be expressed to the collaborators, without whose loyal and painstaking cooperation very little could have been accomplished. Special thanks should also be given to H. W. Rohde for supplying and distributing the samples of beer to the collaborators and to D. A. Coleman for preparing the sample of malt.

REPORT ON WINES

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

No work was done on the topics recommended for study at the last meeting of the Association.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 63 (1937).

The Associate Refereee is convinced that Werder's sorbitol procedure (*Mitt. Lebensm. Hyg.*, **20**, 14) for the detection of added "nongrape material" in grape wine is of doubtful value. Experience with the procedure by the associate referee and by J. B. Wilson was disappointing; recoveries of sorbitol in water solution were low and very erratic. Furthermore, it is now generally recognized that sorbitol is normally present in wines prepared from European grapes (*vitis vinifera*). For these reasons further work on this topic does not seem justified.

Regarding the detection of added water in wine, attention is called to the modern practice of wineries to remove cream of tartar of young wine by chilling. When examined by the "cream of tartar saturation method," wines so treated will indicate added water, even though no water has been added. Obviously the method is not applicable to wines generally.

The recommendation relative to the detection of the use of pomace in wines through a methyl alcohol determination was predicated on the fact that grape pomace contains the main part of the pectin content of the grape and that pectin splits off methyl alcohol during vinification. Accordingly red wines should contain more methyl alcohol than do white wines. A recent investigation (unpublished) showed two commercial Eastern red wines with 31 and 46 mg., and four commercial Eastern white wines showed 4, 4, 4, and 5 mg. of methyl alcohol per 100 cc. Results of the analysis of a large number of California wines did not show the same correlation between the two types of wines; in some cases the white wines showed more methyl alcohol than did the red wines. The aberrant results are due to the fact that in modern cellar practice white wines are frequently made by partial fermentation on the skins. Evidently the occurrence in a wine of an abnormally high methyl alcohol content is not a reliable criterion upon which a charge of adulteration with pomace could be based, and accordingly no further study of the subject is indicated.

Because of the significance of sulfur dioxide, volatile acidity, and alcohol in the grading of wines it is believed that methods for the determination of these ingredients should be studied during the coming year. It may be mentioned that M. A. Joslyn of the University of California has investigated the applicability of the ebullioscope to the accurate determination of the alcohol content of wines. In view of the satisfactory results obtained by Joslyn *et al.*, *This Journal*, **20**, 116 (1937), it is believed that the Association should undertake a study of the instrument.

RECOMMENDATIONS[1]

It is recommended—

(1) That study of the determination of sorbitol, the detection of added water by the cream of tartar saturation method, and the detection of added pomace by a methyl alcohol determination be discontinued.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 63 (1937).

(2) That studies on total sulfur and esterification by lead acetate be continued.

(3) That associate referees be appointed to study the determination of alcohol in wines by the use of the ebullioscope, methods for the determination of volatile acidity in wine, and methods for the determination of sulfur dioxide in wines.

REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the past year the Associate Referee published the results of a critical study of the Enz method for the detection of thujone in absinth-type liqueurs, *This Journal*, **19**, 120 (1936). It was found that the method can be depended upon to give positive tests when 5 mg. or more of the ketone is present, but that a negative test is not conclusive evidence of the absence of thujone. It was found also that the odor of the final steam distillate is an important factor in deciding as to the presence or absence of thujone, since in some cases where a doubtful or a negative test was obtained, the odor of thujone was easily detected by the analyst and furnished organoleptic evidence of the presence of the ketone.

A commercial sample of a liqueur of the absinth type containing about 60 per cent by volume of alcohol and having a strong flavor of anise modified somewhat by the bitter flavor of absinth, was examined in the laboratories of the Food and Drug Administration by the method described, with the results given in Table 1.

TABLE 1.—*Thujone by Enz method in absinth-type liqueur*

COLLABORATOR	BOTTLE NO.	THUJONE ODOR	THUJONE TEST
C. A. Wood	1	—	positive
	2	positive	negative
J. B. Wilson	3 to 11	positive	positive
	12	doubtful	doubtful
R. A. Osborn	composite*	positive	positive
	composite*+5 mg. thujone	positive	positive

* The composite sample consisted of a mixture of bottles 3 to 7, inclusive.

Osborn stated that the sample to which thujone had been added had a stronger odor of thujone and gave more color in the test.

The collaborative results obtained on the commercial sample agree with those reported by the Associate Referee in the paper referred to previously.

It is recommended[1] that the method for detection of thujone discussed in this report be adopted as tentative.

No report on whiskey, rum, and brandy was given by the associate referee.

No report on fusel oil was given by the associate referee.

No report on methyl alcohol was given by the associate referee.

J. W. SALE: The laboratories of the Bureau of Internal Revenue and the Bureau of Customs are greatly concerned with the analysis of liquors. We have with us today Dr. H. J. Wollner, Consulting Chemist to the Secretary of the Treasury, and we would appreciate very much a few words from Dr. Wollner regarding the work of those laboratories.

Dr. WOLLNER: I am very grateful to Mr. Sale for this opportunity of presenting a matter that is giving us a considerable amount of concern. In prefacing my remarks I should like to say that there is nothing definite to be drawn from them, nor are they intended to refer to our present A.O.A.C. methods, as we recognize the fact that the present methods are not predicated upon and designed necessarily for the specific use of the Treasury Department. The Treasury Department is burdened with the responsibility of the administrative control of the alcoholic industry as such and particularly of the alcoholic beverage industry. It has also the burdensome problem of applying and collecting a tax from materials which today, in 1936, are still a very mystical quantity. By that I mean that, collectively or individually, no one can define whiskey in purely technological terms. Nevertheless, $500,000,000 annually is the revenue which the Government derives from this commodity. What are we after? We are searching for critical procedures, not methods or procedures that will give us an approximate generalized appreciation of the liquid we have in our hands, but which will discriminate between an alcohol derived from corn and an alcohol derived from rye. We are interested in discriminating between alcohol from synthetic processes and alcohol derived from cereals, from fermentation in other words. Today such procedures, though very vital to the Treasury Department, are not available. Under these circumstances the technical staff of the Treasury Department is essaying an entirely new approach to the problem.

I do not intend to burden you with the details of what we are doing. Mr. Schicktanz will present some of the technical developments that it has been our fortune to make in the laboratory during the past year. I

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 63 (1937).

should like to say that we most cordially invite you to consider the problem and to cooperate. We are spending a great deal in time, effort, personnel, and equipment to lay the basic considerations. What are these? Our present methods fail to approach the thing except in a highly empirical fashion. Our methods for determination of esters, acids, higher alcohols, etc. are predicated on a traditional background and not upon the developments of any one or several laboratories. It seems to us, therefore, that in our approach our first step shall be, and it is at the present time, to take a whiskey derived from a known source, known procedure and technic and by methods which we are now developing reduce it to its elementaries. We are working with modern laboratory equipment that hitherto has not been very proposedly applied to whiskey. We are happy to report that we are making progress. I hope Mr. Schicktanz will be able to place sufficient information before you to give evidence of that fact and invite your further study of the problem.

There has been a considerable amount of criticism of the A.O.A.C. procedure in the past, not only by ourselves but by the members of the industry. They have felt, for example, that our fusel oil methods have been, shall I be charitable and say, wanting. We in the Treasury Department have recently stopped all work along that line. We felt that we were merely piling up more and more statistics, which would be not only misleading but would defeat our ultimate purpose. Once having established the elementals we are dealing with, it may be possible for us to develop short cuts—short, simple, routine control tests, which will make for a comprehensive control of the general problem. At the present time we have these tests, the accuracy of which we question in some cases, and which we know are inaccurate in other cases. We should like your help.

J. W. SALE: We are very appreciative of these remarks by Dr. Wollner, and certainly they mean to me that the Association, in its work on alcoholic beverages, cannot get along without the cooperation of the Treasury Department laboratories, and on the other hand I feel that the method of approach which is historic with this Association, that is, the collaborative testing out of methods, should be of great value in the work that Dr. Wollner has undertaken. We shall appreciate any recommendations that the laboratories of the Bureau of Internal Revenue and the Bureau of Customs may make with respect to the future trend of our collaborative work in testing out methods for alcoholic beverages, and I trust that the members of those laboratories will feel free to suggest to us lines of approach for our future work.

J. W. SALE: This is an appropriate time to have presented a paper which does not appear on the program, the title of which is "Titration Curves of Whiskey and Their Analysis," by S. P. Schicktanz. This paper was published in *Ind. Eng. Chem.*, **29**, 157 (1937).

The paper entitled "Studies on Proteolytic Activity of Barley Malt," presented by Stephen Laufer, will be found on p. 307 of this number of *This Journal*.

A paper, entitled "Regulation of the Alcoholic Content in Beer," by Carl Rach, Staten Island, N. Y., was presented by J. B. Wilson. This paper will not be published in *This Journal*.

A paper, entitled "A Comparison of Several Physical Methods for the Determination of the Alcohol Content of Wine," by M. A. Joslyn, G. L. Marsh, and J. Fessler was presented by R. A. Osborn. The paper has been published, *This Journal*, **20**, 116 (1937).

J. W. SALE: We are indeed very appreciative of the work of Dr. Joslyn and his associates in presenting the results of this research on the determination of alcohol in wine. We do not use the ebullioscope or ebulliometer in the Food and Drug Administration, therefore I cannot comment on it, but I understand that it is in general use in the laboratories of the Bureau of Internal Revenue and perhaps also in the laboratories of the Bureau of Customs. I should appreciate having a word or two about this instrument from Mr. Peter Valaer, of the Bureau of Internal Revenue.

PETER VALAER: My first experience with the ebullioscope was many years ago when we had to translate all the details from the French to put it into use. We were surprised to find it was as good as it was. Now our Department has been very successful in its use; in fact, some of our members have obtained amazing results. However, we find that sometimes the method breaks down. Certain substances like essential oils will give results out of line, and volatile material, such as ginger and certain aromatics, affects the results. High solids will sometimes send the results up and sometimes down. The best results with the ebullioscope are obtained on distillates, if they are carefully made, as with a pycnometer. Of course, the instrument is at the mercy of the thermometer. The thermometer should be good. Ours are tested by the Bureau of Standards and consequently we get good results. Before recommending the use of the ebullioscope as official for the A.O.A.C. I think much more work should be done. The original makers of the ebullioscope limited its use. Some insisted it was intended for beer and others insisted it was intended for wine. But we have extended its use to distilled liqueurs and for other purposes whenever it is convenient. Mr. Ryan, a chemist in our office, has devised tables in which the alcoholic content of preparations containing excessive alcohol can be determined. It is a fine piece of work, but we should prefer to do more work before we recommend the use of the ebullioscope.

MONDAY—AFTERNOON SESSION

REPORT ON EGGS AND EGG PRODUCTS

By H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The associate referees' reports this year include no recommendations for the adoption of methods. A report of progress is given by Joseph Callaway, Jr., on methods for the detection of decomposition, with a supplementary report by Manuel Tubis on methods for ammonia nitrogen. E. O. Haenni, Associate Referee on Unsaponifiable Matter and Fat, reports progress but will present no formal report. Studies should be continued on these subjects, as well as on the others on which no reports are offered.

One error has been found in the chapter, Eggs and Egg Products, *Methods of Analysis*, 1935. The reference in Section **28**, page 305, should read **XX, 79.**

It is recommended[1]—

(1) That studies be continued on methods for the determination of acidity of the lipoids (rapid method) and identification of the acid-reacting substances, ammonia nitrogen, acid-soluble phosphoric acid, and any other methods for demonstrating decomposition.

(2) That studies of methods for the determination of cholesterol and fat be continued.

(3) That studies of methods for the determination of sugar, added salt, and glycerol be continued.

(4) That studies be continued on the method for water-soluble and crude albumin nitrogen in dried eggs.

No report on unsaponifiable constituents and fat was given by the associate referee.

REPORT ON DECOMPOSITION IN EGGS

By JOSEPH CALLAWAY, JR. (U. S. Food and Drug Administration, New York), *Associate Referee*

In reporting to the Association last year, this Associate Referee called attention to four types of decomposition products, one or more of which might be expected in liquid eggs that had undergone decomposition, and suggested that there should be available chemical methods for the detection and determination of such products of decomposition.

The Association has adopted a method for determining the so-called

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 59 (1937).

acidity of the fat; it is now known as the determination of the acidity of the ether extract. The amount of acid-reacting substances in the ether extract is indicative of decomposition of the fat in eggs and possibly also of the lecithin. Last year the Associate Referee reported on some collaborative work done on a method for the determination of ammonia nitrogen, which is indicative of the amount of protein decomposition that may have occurred. The method studied had previously been considered by the Association, but no collaborative work had been done. As a result of last year's work, the method was adopted as tentative.

Since that time there has appeared a paper by Bandemer and Schaible,[1] who report work done on a method for determining ammonia nitrogen in liquid eggs by absorbing the ammonia in dilute acid without aerating the eggs. This method is not nearly so time consuming as the tentative method studied last year.

At the suggestion of the Associate Referee, Manuel Tubis of the Philadelphia Station of the Food and Drug Administration, who was making determinations of ammonia nitrogen in certain types of shell eggs, made similar determinations by the method of Bandemer and Schaible. A report by Mr. Tubis on this work has been received by the Association and will be published as a separate report.

Tubis shows that in the eggs examined by him, comparable results were obtained by the two methods in most instances. Further work to determine if the Bandemer and Schaible method is preferable to the tentative method should be done. It must be borne in mind, however, that many data have been collected on the ammonia nitrogen content of eggs as determined by methods essentially the same as the present tentative method.

The official method for the determination of acidity of the ether extract is rather time consuming. A rapid method applicable to dried eggs was studied a few years ago and last year the General Referee recommended that this rapid method be further studied. Before further time is given to a study of dried eggs, it was thought desirable to see if a rapid method could be used for liquid eggs.

The fat obtained by the acid hydrolysis method for total fat cannot be used to determine the acidity of the fat in the liquid egg since the material recovered after acid hydrolysis contains considerable amounts of free fatty acid. This acid probably comes from decomposition of the lecithin by the strong hydrochloric acid used for hydrolyzing the egg proteins.

Various methods of extracting the unchanged lipoids of eggs have been tried. After some preliminary experiments, the Associate Referee decided that extraction of the lipoids by a mixture of ether and petroleum ether after treating the liquid egg with a solution of sodium chloride in dilute alcohol probably gave as satisfactory results as any other rapid extraction

[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 201 (1936).

method. Complete recovery of the lipoids cannot be obtained unless several extractions with the mixed ethers are made. However, since from 75 to 80 per cent of the total lipoids is extracted by two shake-outs with the mixed ethers, it was decided to use these lipoids for determination of acidity. The actual acidity was found by titrating the lipoids dissolved in neutral benzene with 0.05 *N* sodium ethylate. The following procedure was used:

Prepare an aqueous alcoholic salt solution by dissolving 10 grams of salt in about 50 cc. of water, adding 30 cc. of 95% alcohol and diluting to 100 cc. with water.

Weigh out about 10 grams of mixed whole liquid egg or 5 grams of liquid yolks. Transfer to a suitable centrifuge bottle with 40 cc. of the above salt solution. Shake gently until the egg is thoroughly mixed with the salt solution. Now add 50 cc. of ethyl ether followed by 50 cc. of petroleum ether, stopper the bottle, and shake gently but thoroughly until an emulsion begins to form. Centrifuge to separate the liquids. If a bad emulsion has formed, add 10 cc. of ethyl alcohol, shake gently, and centrifuge again. Remove the mixed ether layer by carefully pouring off or by blowing off with a wash bottle arrangement or any other manner that will separate ether layers. Repeat the extraction, using 30 cc. each of ether and petroleum ether, and add to the first ether shakeout. If only acidity of the ether extract is to be determined, enough lipoids for titration purposes will be extracted by two extractions. If a complete extraction is desired, repeat the shaking with about 50 cc. of mixed ethers, centrifuging, etc., until the ether layer is colorless.

Evaporate the first two extracts or the combined extracts, if desired, in a suitable dish on the steam bath. When the ether is removed, add 5 cc. of absolute alcohol and evaporate, to aid in the removal of moisture. Now dissolve the residual extract in a small amount of $CHCl_3$, filter into a tared beaker, and wash the flask and filter with $CHCl_3$. Evaporate off the $CHCl_3$ on the steam bath and continue heating a few minutes after the $CHCl_3$ is removed. Dry the beaker with a towel. Cool, and weigh.

Dissolve the residue in the beaker in neutral benzene and titrate with 0.05 *N* sodium ethylate to phenolphthalein as in the official method.

Report acidity as cc. of 0.05 *N* sodium ethylate for each gram of ether extract.

This procedure was tried by the Associate Referee on a few samples of eggs in comparison with the present official method. Collaborators were requested to obtain samples of good and bad eggs and determine acidity of ether extract by both of these methods. The results obtained are as follows, expressed as cc. of 0.05 *N* sodium ethylate per gram of lipoids:

SAMPLE	OFFICIAL METHOD	PROPOSED RAPID METHOD
Results by Associate Referee		
Commercial Shell Eggs	1.6	1.6
Addled Eggs	2.3	2.5
Mixed Rots	3.4	3.3
Results from C. D. Schiffman, F. & D. Adm., Atlanta, Ga.		
1a*	0.96	1.04
1b	0.97	0.97
2a	0.98	1.02
2b	0.98	0.77

3a	2.35	1.53
3b	2.66	1.48
4a	1.10	1.15
4b	1.26	0.84

* 1a & b, duplicate determinations on fresh eggs.
2a & b, duplicate determinations on lowest grade commercial eggs.
3a & b, duplicate determinations on inedible but not putrid eggs.
4a & b, duplicate determinations on incubated 21 days, infertile eggs.

Comments by Schiffman.—The bad eggs in this lot did not give as high an acidity of the fat as might be expected and I am holding same in refrigerator in hopes of finding time to repeat this work and see what would be the results in case of higher acidity of fat. I find the proposed rapid method quicker, but apparently not quite so accurate as the official method. I did not obtain as good checks in the rapid as in the official method but for sorting purposes it would be quite useful. Note especially the variation between methods in Sample 3.

Results from H. I. Macomber, F. & D. Adm., Baltimore, Md.

SAMPLE	OFFICIAL METHOD	PROPOSED RAPID METHOD
Good Eggs	2.4	1.8
	2.3	1.6
Bad Eggs	3.1	2.2
	2.7	2.3

Comments by Macomber.—Since the eggs used for "bad" eggs were really borderline, the results obtained this time by the proposed method seem to be much better than those obtained by the A.O.A.C. method. I cannot account for the apparently high results obtained by the A.O.A.C. method. The only apparent deviation from the method was the use of a Soxhlet extractor instead of the Knorr type.

Results from Samuel Alfend, F. & D. Adm., St. Louis, Mo.

SAMPLE	OFFICIAL METHOD	PROPOSED RAPID METHOD
Good Eggs	1.85	2.05
	1.85	2.00
Bad Eggs	3.75	3.82
	3.65	3.90

Comments by Alfend.—Two lots of eggs were obtained at a breaker; one was of passable quality and the other consisted of rejects such as white rots, addled eggs, and so on. These latter were quite bad in character but contained no black rots.

I encountered no difficulties with the proposed rapid method. However, I have a prejudice against any extraction method that involves pouring or blowing the lighter liquid off the top. I do not see the advantage in using the ethyl ether-petroleum ether solvent as against the chloroform-alcohol solvent used by Mitchell.

The results reported show fairly close correlation between the rapid method and the official method.

It is recommended[1] that further study be made of a rapid method for determining acidity of lipoids, and also that some study be made of the identity of the acid-reacting substances and of methods for determining other decomposition products.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 59 (1937).

REPORT ON AMMONIA NITROGEN IN EGGS

By MANUEL TUBIS (U. S. Food and Drug Administration, Philadelphia, Pa.)

Certain types of shell eggs were being examined in this laboratory for ammonia nitrogen by the method given in U. S. Dept. Agr. Bull. 846 (now tentative A.O.A.C. method). At the request of the Associate Referee on Decomposition in Eggs, determinations of ammonia nitrogen were made on portions of some samples by the method of Bandemer and Schaible.[1]

The slight modifications made in their method pertain to the construction of the cell and the time required for absorption. In the modification used by the writer the inner chamber of the cell consists of a small Petri dish, temporarily held in place by grease, instead of the glass ring cemented to the floor of the Petri dish, which is difficult to make, maintain, and clean. A further disadvantage is that the end point of the indicator is difficult to see due to the dark red-brown cast caused by the de Khotinsky cement. This is obviated by the white opalescence of the grease.

In the modification, the 5 gram sample can be weighed conveniently directly in the cell, obviating any specific gravity determination for calculation to the 100 gram basis.

The inner chamber consists of a small Petri dish, 50 mm. outside diameter$\times$10 mm. inside height; 5 cc. of 0.005 *N* acid, containing the indicator and made according to Bandemer and Schaible, is placed in the inner chamber. After "incubation" the excess acid is titrated with 0.0025 *N* alkali to the point of least color of the indicator. The quantity of acid can be varied within narrow limits, as indicated by the condition of the egg; however, the total volume of liquid in the inner chamber must not be more than 15 cc. One cc. of acid is equivalent to 0.07 mg. of nitrogen or 0.085 mg. of ammonia.

The time required for liberation and absorption was longer than that given by Bandemer and Schaible, but still much less than the time required in the A.O.A.C. method. Recoveries when ammonium sulfate solution was used and ammonium sulfate was added to the eggs were better than 97 per cent.

Results on known solutions of ammonium sulfate obtained by the modified Bandemer and Schaible method are given in Table 1.

Comparative results on weak eggs are given in Table 2. The eggs were broken into Petri dishes, and the physical appearance was noted; they were then transferred to small flasks, glass beads were added, and the whole egg was mixed. Determinations of ammonia nitrogen were made on 25 gram aliquots according to the A.O.A.C. method and also by the

[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 201 (1936).

TABLE 1.—*Results on known solutions by modified method*
(Results expressed as mg. of ammonia nitrogen per 100 grams.)

TIME AT 37.0°± 0.5° C.	PRESENT	RECOVERED	RECOVERY
hours			*per cent*
¾	3.75	0.96	25.6
1½	3.79	1.38	36.4
2¼	3.81	1.99	52.2
3	3.74	3.17	84.8
3¾	3.79	3.59	94.7
4¼	3.68	3.73	101.4
4½	5.91	5.89	99.7

modified method described. Aliquot parts of 5 grams were used and results were calculated to 100 grams basis. Absorptions were at least 5 hours long.

TABLE 2.—*Results on weak eggs*

(RESULTS EXPRESSED AS MG. OF AMMONIA NITROGEN PER 100 GRAMS.)

EGG NO.	PHYSICAL APPEARANCES	A.O.A.C. METHOD	MODIFIED METHOD OF BANDEMER AND SCHAIBLE
1	Membranes gone, blood spot, white very watery, odor normal	2.99	
2	Membranes gone, white very dark in color, normal odor	1.47	1.66, 1.85*
3	Membranes broken, contents mixed, slight odor	3.80	3.77
4	Yolk flat, some membranes intact, slight odor	3.30	4.13
5	Membranes partly broken, normal odor	2.51	3.29, 3.44*
6	Membranes partly broken, normal odor	4.05	5.30
7	Stuck yolk, yolk mixed and fibrous, normal odor	1.76	
8	Stuck yolk, yolk mixed and fibrous, normal odor	2.15	2.08, 1.89*
9	Yolk membranes gone and yolk mixed, normal odor	3.74	4.80
10	Yolk membranes gone and yolk mixed, normal odor	1.90	1.88
11	Yolk "clotted" and somewhat mixed, slightly bad odor	3.56	2.94
12	Yolk mixed, normal odor	1.78	2.28
	Fresh whole egg		1.61

* Duplicates run at later date

DISCUSSION

The results obtained by the modified Bandemer and Schaible method are generally higher than those obtained by the A.O.A.C. method. This is accounted for in part by the smaller sample, the increased recovery, and the final calculation.

The advantages of the new method are simplified technic, shorter time required, less complicated and expensive apparatus, and better recovery.

No report on glycerol, sugar, and added salt was given by the associate referee.

No report on dried eggs was given by the associate referee.

REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

The work on preservatives was somewhat delayed owing to the withdrawal of the former referee, which necessitated reassignment of the subject during the past year. At the suggestion of the Chairman of the Committee on Recommendations of Referees a study of the recently adopted method for the determination of saccharin in non-alcoholic beverages was begun, to determine whether or not it may be adapted to the other foods listed in the present official method.

A sample of apple butter containing 278 p. p. m. of sodium saccharin equivalent to 200 p.p.m. of the insoluble form was employed. The sample was prepared as directed in *Methods of Analysis, A.O.A.C.*, 1935, **XXXII, 12(c)**, p. 434, and extracted and tested essentially by the method for the determination of saccharin in non-alcoholic beverages described in **XXXII, 15**, p. 435 of the same publication. While it is believed that the saccharin is quantitatively extracted, it was difficult to make the color comparison because of a cloudiness that developed almost immediately in the sample tube. Several modifications, such as increased washing of the ether extract and filtration of the hydrochloric acid hydrolysate, were tried, but they failed to remove the interfering substances.

It is recommended[1] that further work be done on the method for the determination of saccharin in non-alcoholic beverages to learn whether or not it is applicable to the other food products listed in the official method.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

The collaborative problem undertaken this year was the estimation of ponceau SX in the presence of ponceau 3R. The Referee sent five sets

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 64 (1937).

of samples, consisting of four subdivisions each, to five collaborators with the following instructions and method to estimate the dyes:

QUANTITATIVE ESTIMATION OF PONCEAU SX IN PRESENCE OF PONCEAU 3R

The method is based on the observation that ponceau 3R can be oxidized in an alkaline medium, whereby it will be completely destroyed. Ponceau SX, on the other hand, being much more resistant to this treatment, is affected only to a slight extent. Since the amount of ponceau SX destroyed appears to be proportional under definite conditions, quantitative results can be obtained by applying a given factor.

EXPERIMENTAL PART

Solutions required

Hydrogen peroxide	3% (10 vol.)
Sodium hydroxide	10%
Sulfuric acid	1 +4
Ammonium acetate	1 +1

Prepare a 1% solution of the dye mixture. Titrate 20 cc. portions of above dye solution with 0.1 N Ti_2Cl_6, using Na citrate as a buffer. Avoid over titration as it will give erroneous results. Note results obtained. Mark a number of 300 cc. Erlenmeyer flasks with a suitable agent (crayon, pencil, or glass cutter) at a volume of 50 cc. Pipet that amount of the dye solution into the marked flasks, which is equivalent to 12–15 cc. of the standard Ti_2Cl_6; dilute with sufficient water to make 70 cc. and add in the order named, 8 cc. of H_2O_2 and 2 cc. of NaOH. (The total volume in the flask should measure finally 80 cc.) Introduce also into the flask a perforated Pt disk or a Pt wire spiral to act as a contact agent. Cover the flask with a short-stemmed funnel and boil vigorously over asbestos until the 50 cc. volume mark is reached. Remove the flask from flame, cool, add 25 cc. of alcohol, and evaporate over a live steam bath until the 50 cc. mark is reached. Remove the funnel from flask during evaporation. Dilute the contents with 100 cc. of water, and add 5 cc. of NH_4 acetate, 1 cc. of H_2SO_4, and 10 grams of Na citrate. Boil, and titrate until a colorless or a yellowish solution is obtained. Multiply the number of cc. required to reduce the ponceau SX by 1.167, the result representing the value for the latter dye. The difference between the total dye titration and the corrected value is due to Ponceau 3R.

1 cc. of 0.1 N $TiCl_3$ – 0.012360 gram of ponceau 3R
0.012006 gram of ponceau SX.

The samples in question were of the following composition (evaluated by titration with titanium chloride):

Sample No.	*per cent*
1	Ponceau SX = 21.26
	Ponceau 3R = 62.75
2	Ponceau SX = None
	Ponceau 3R = 83.67
3	Ponceau SX = 63.78
	Ponceau 3R = 20.91
4	Ponceau SX = 85.04
	Ponceau 3R = None

The reports of the collaborators follow:

	Sample No.	per cent
O. L. Evenson	1	Ponceau SX = 22.2
F. & D. Adm.,		Ponceau 3R = 62.4
Washington, D. C.	2	Ponceau SX = Trace or None
		Ponceau 3R = 84.0
	3	Ponceau SX = 73.2
		Ponceau 3R = 11.1
	4	By direct titration as Ponceau SX = 85%; if correction factor is applied, Ponceau SX = 100.0%. Therefore Ponceau 3R = Absent
S. S. Forrest	1	Ponceau SX = 22.80
F. & D. Adm., Washing-		Ponceau 3R = 60.80
ton, D. C.	2	Ponceau SX = Trace
		Ponceau 3R = 83.6
	3	Ponceau SX = 72.0
		Ponceau 3R = 12.3
	4	By direct titration as Ponceau SX = 85%; if correction factor is applied, Ponceau SX = 100.0%. Therefore Ponceau 3R = None
J. T. Scanlan	1	Ponceau SX = 22.51
Bur. of Chemistry and		Ponceau 3R = 63.22
Soils, Washington, D. C.	2	Ponceau SX = Doubtful
		Ponceau 3R = 85.65
	3	Ponceau SX = 69.57
		Ponceau 3R = 17.06
	4	By direct titration as Ponceau SX = 86.86%; if correction factor is applied, Ponceau SX = 94.07%. Therefore Ponceau 3R = None
J. L. Hogan	1	Ponceau SX = 20.47
F. & D. Adm., New		Ponceau 3R = 64.77
York, N. Y.	2	Ponceau SX = None
		Ponceau 3R = 84 36
	3	Ponceau SX = 66.36
		Ponceau 3R = 19 47
	4	By direct titration as Ponceau SX = 86 32%; if correction factor is applied, Ponceau SX = 93.05%. Therefore Ponceau 3R = None.
F. J. Hope	1	Ponceau SX = 26.0
H. Kohnstamm, Brook-		Ponceau 3R = 59.9
lyn, N. Y.	2	Ponceau SX = 2.9
		Ponceau 3R = 82.6
	3	Ponceau SX = 73.2
		Ponceau 3R = 12.2
	4	Ponceau SX = 100.8
		Ponceau 3R = 13.9

The following comments and criticism were given by the collaborators:

O. L. Evenson.—Some high results for ponceau SX and correspondingly low results for ponceau 3R were obtained on all the samples except No. 2. The high figures obtained were apparently due to reducible material, probably H_2O_2, left after the final treatment. This was indicated by the liberation of iodine after suitable treatment.

S. S. Forrest.—Apparently some reducible substance, probably H_2O_2, tends to remain after the final treatment. This would account for the high results obtained with No. 4. Considerable difficulty was encountered in obtaining check values on the titrations after the final treatment. This seems to make accurate determinations very difficult.

J. T. Scanlan.—Sample No. 1, no comment. Sample No. 2: After the oxidation no red color could be observed in the solution. Titration with $TiCl_3$ caused a slight change in color, but since it seemed doubtful that the consumption of 0.3 cc. of 0.1 *N* $TiCl_3$ was due to ponceau SX, the sample was calculated as containing ponceau 3R. Sample No. 3, no comment. Sample No. 4: In view of the results obtained with this mixture it appears that as described the method leaves some reducible substance other than the original dye present, and that this reducible material reacts before the latter reduces the dye present and gives erroneous results. Presumably the same error occurs in all the samples.

J. L. Hogan.—Samples No. 1 and No. 3 are undoubtedly dye mixtures. Sample No. 2 apparently consists of ponceau 3R only, since it decolorizes completely with an alkaline H_2O_2 solution. The real difficulty arises in sample No. 4. In every instance the titration of the treated material multiplied by a given factor results in a number larger than the first titration. It is my opinion that the factor cannot be regarded as constant over the entire range of mixtures of the dyes.

F. J. Hope.—From my results it is evident that there is a large error in sample No. 4.

DISCUSSION

A summary of the results obtained by the collaborators shows that fairly good results were recorded with sample No. 1. A maximum deviation of 1.5 per cent from actual value was reported by four of the five collaborators. In sample No. 2, which consisted of ponceau 3R only, four of the five collaborators agreed that either there was no ponceau SX present or it was only present in traces.

With sample No. 3 serious trouble apparently was experienced. While all collaborators agreed that both dyes were present, the amounts reported exceeded the actual quantity by over 9.0 per cent. As to sample No. 4, which consisted of ponceau SX only, four of the five collaborators agreed that it only consisted of this dye, but the factor used for the treated sample did not agree with the original titration, being in every case in excess.

As was observed by one of the collaborators that established the presence of undecomposed hydrogen peroxide in the treated mixture, the errors are probably due to this substance. It is imperative, therefore, that the excess of hydrogen peroxide be eliminated if quantitative results are to be expected. With this end in view the Referee desires to

send out additional mixtures of these two dyes, with a modification of the present method.

The Referee wishes to state further that investigational work is at present being conducted on the separation and quantitative estimation of sunset yellow FCF from tartrazine.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be continued on the quantitative estimation of ponceau SX and ponceau 3R.

(2) That investigational work be continued on the quantitative estimation of sunset yellow FCF in the presence of tartrazine.

(3) That investigational work be undertaken to separate and estimate quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

(4) That the method submitted for extraction and identification of added color in macaroni and similar products[2] be made official (final action).

REPORT ON METALS IN FOODS

By H. J. Wichmann (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

ARSENIC

The salient points of interest on the arsenic project this year are the completion of the work on methods of preparation of sample and the introduction[3] of the idea of isolating arsenic by extraction of arsenic ethyl xanthate with non-aqueous solvents, followed by oxidation to arsenic acid and determination by the production of the molybdenum blue color according to Zinzadze.

The work on sample preparation was confined to shrimp and tobacco, probably the two most striking examples of difficult sample preparation available. The arsenic in shrimp exists largely in organic combination, and must be first transformed quantitatively into the inorganic form. Tobacco is a troublesome product to analyze for arsenic because the pyridine residue from nicotine resists complete digestion and inhibits the arsine evolution. The closed systems of burning in oxygen are effective in overcoming these troubles, but the necessary apparatus is expensive and not always readily available, and the operation is exacting and time-consuming. Practical substitutes were therefore sought. Ordinary digestion with nitric and sulfuric acid, followed by perchloric acid digestion,

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 64 (1937).

[2] *Methods of Analysis, A.O.A.C.*, 1935, 229.

[3] An abstract of a paper by Klein and Vorhes was given at the 1936 meeting. The detailed paper will be published in a later number of *This Journal*.

destroys refractory organic matter and transforms all the arsenic into the inorganic state. Cassil's results on shrimp and tobacco are very good.

The system of ashing with nitric acid and magnesium nitrate as fixatives was likewise successful with these two products. Since shrimp, tobacco, cod-liver oil and resinous woods are the most refractory of all arsenical materials encountered by the referee in recent years, their conquest should indicate successful handling of other material. After the confirmation of Cassil's results, the subject of sample preparation may be considered closed. Cassil's idea of strip impregnation in a vacuum and selection of dense granulated zinc may be the means of extending the range of the Gutzeit method in the near future, which is greatly to be desired.

The Referee is pleased with the Klein-Vorhes idea of transforming arsenic into an organic combination soluble in non-aqueous solvents. This system of isolation has been particularly successful in the various dithizone methods for lead, mercury, etc., in the carbamate method for copper, and in the determination of many other elements. At present the sponsors have a number of details to iron out and clarify. After the isolation the arsenic can, of course, be determined by a number of different methods. The Referee appreciates the work done by Klein and Vorhes on the molybdenum blue method of arsenic determination and believes that it should be continued, because this method seems to be a possible official method. It lends itself particularly well to photometric or electrophotometric methods of measurement, and the solubility of the blue color in certain organic solvents should be useful in the determination of a few gamma of arsenic.

The Referee would also repeat his last year's recommendation that the arsine evolution method of arsenic isolation be given attention next year. In his opinion the greatest probability of success with the arsine evolution is as a method of isolation, and it should not be tied up with any particular method of determination. After an arsine evolution the analyst should be able to choose the manner of determination according to circumstances. In laying stress on methods of sample preparation, isolation, and final determination of the arsenic, the Referee has in mind the formulation of a flow sheet for arsenic similar to that for lead given in the new 4th Edition of *Methods of Analysis, A.O.A.C.*

It is the understanding of the Referee that tartar emetic is now used to combat various fruit flies under semi-tropical conditions. Success here would insure the use of antimony compounds under other conditions, and the element would no longer be a negligible factor in spray residue considerations. During the coming year the Referee expects that some antimony methods suitable for spray residue work or for even smaller quantities will be published. The growers may mix antimony with arsenic in more or less shotgun mixtures if antimony compounds are found to be effective

against insects. Therefore more attention must be paid to the problem of interferences in the determination of either element and a method of separating very small quantities of arsenic and antimony must be provided. This work is recommended to future associate referees. It is expected that next year may bring a recommendation for the appointment of an Associate Referee on Antimony to make up for past neglect of this element.

COPPER

The Associate Referee restricted his work this year to the extractive carbamate method for copper. Organic-complex formation of metals followed by extraction by non-aqueous solvents has proved so successful in other determinations, notably lead, that it is small wonder analysts are turning to this principle in greater numbers. The success of this year's work seems to indicate that in the case of copper the doubtful preliminary sulfide precipitation can be dispensed with. According to the Associate Referee's report, bismuth and, to a small extent, manganese, offer the only serious interference. The maximum errors on 10–100 gamma of copper were 14.6 and 19.5 per cent, which is more than the error of the dithizone method for lead. It is the Referee's opinion that greater accuracy over even smaller ranges will depend upon a change of instruments. He therefore recommends that the next associate referee turn to photometric measurement with color filters. An absorption curve of the copper complex should locate the optimum wave-length for the filter. An absorption curve for the bismuth complex, the only serious interference, should also be obtained. If it is impossible to overcome the interference optically, the next associate referee should pay some attention to *p*H control or complex formation as methods for the elimination of the bismuth interference. The manganese pyrophosphate complex seems to take care of the interference of that element. The Referee believes that the carbamate method for copper has an advantage over the dithizone method because (1) the carbamate reagent is colorless, (2) carbamates other than copper, bismuth, and manganese are colorless, (3) carbamates insoluble in water are either soluble in carbon tetrachloride or other solvents with colorless solutions, or are attracted to the interface and thus can be separated from the copper solution. The two methods may be of equal sensitivity, but the problem of interference in the carbamate method is probably simpler. Other problems are the determination of the optimum concentration of citrates to take care of reasonable amounts of phosphates, the optimum *p*H of extraction, especially in the presence of iron and phosphates, and the best solvent for the complex. Naturally the one with the greatest solubility is desired to insure the best partition. It may be possible to recommend a method for adoption as tentative next year, therefore it is suggested that the work on copper be continued.

FLUORINE

Collaboration on the determination of fluorine in calcium phosphates developed the fact that the critical factors governing the Willard and Winter distillation have not yet been fully appreciated. Until analysts generally can make the proper distillation, there is very little use in worrying about the final determination. When the Associate Referee had the opportunity to work with some of those analysts whose results showed the greatest "scatter," and to show them the various pitfalls in the distillation, the results, graphically expressed, approached a normal distribution curve. Last year the Associate Referee presented data showing the influence of input volume, temperature, and volume of distillate on recovery with sulfuric acid as the volatilizing agent. This year he made a similar study with perchloric acid and ortho-phosphoric acid. His results indicate that even greater care is necessary to obtain good recoveries in the distillation with these two acids. It is essential, therefore, that analysts have information as to what constitutes a proper distillation before they can be expected to make 100 per cent recoveries.

The Referee wishes to take due notice of the recently published work of Armstrong on the titration of gamma quantities of fluorine. Previous titration methods have not determined quantities as small as 1 or 2 gamma. Armstrong has eliminated the use of alcohol, and concentrated his volume from 40 to 2 cc. The sensitivity of colorimetric methods can be likewise increased by greatly diminishing the volume. No doubt Armstrong's idea should be checked up next year.

Analysts interested in fluorine in water or foods have been proceeding on the theory that the fluorine exists therein as an inorganic compound, but the Referee desires to leave the thought that fluorine might exist in some food products as an organic compound analogous to arsenic in shrimp. If this supposition should be a fact, there will still be the problem of determining such fluorine and perhaps differentiating it from the inorganic variety. This would be another problem in sample preparation. Sample preparation in general is certainly of the utmost importance, and therefore it is recommended that next year greater stress be placed on this phase of the work. Any analysts especially interested in the determination of fluorine in organic matter are urged to extend all possible help to the Associate Referee.

LEAD

In the field of lead investigations the developments of interest during the year were the publication of the paper, "Dithizone Methods for the Determination of Lead," the preparation of the lead methods included in the 4th Edition of *Methods of Analysis*, and the construction of 16 photometers for distribution in the field laboratories of the Food and Drug Administration. The ground has therefore been prepared for some

good collaborative work on lead methods next year. Special topics recommended for study are sample preparation for oils and baking powder, and especially simplification of interference removal in the lead determination. The Referee had hoped to be able to call attention to publication of data on equilibrium curves for metals such as bismuth, stannous tin, copper, and zinc with dithizone. A few individuals have expressed interest and an intention to do something, but no information has been received of any accomplishments. However, *p*H control should greatly assist in establishing new dithizone methods and elimination of interferences.

MERCURY

The Associate Referee presented no report. Last year's recommendation of continued work on dithizone mercury methods is therefore repeated, with the added recommendation that photometric methods especially be studied.

SELENIUM

The Associate Referee's report indicates that ashing methods with fixatives have not been studied sufficiently to indicate whether they are possible or practicable. It is recommended that some answer to this question be supplied next year. A system of wet digestion with nitric acid, sulfuric acid, and a catalyst under a trap or reflux is the Associate Referee's idea of the best system of sample preparation in use at present. Partial digestion in an open system at a minimum temperature may cause slight losses, but the errors of the turbidimetric method of determination used in this study may be equally large, so that really a more sensitive method is necessary to settle the question. The Associate Referee and his assistants appear to have found such a sensitive method in the electrometric titration with thiosulfate solutions. Therefore they should be able to evaluate preparations satisfactorily next year. Apparently there is no difference of opinion about the reliability of the distillation and precipitation system of selenium isolation. Therefore the remaining problem is that of the method of determination. The Associate Referee presents some interesting comparative data on the per cent errors of the turbidimetric and volumetric-electrometric systems of determination. It is unlikely that errors less than 10–15 per cent can ever be produced turbidimetrically. The volumetric method in the 1–10 gamma range produces good results, but the percentage error in the lower part of the range is at present still greater than the Association desires. With larger quantities of selenium the accuracy is excellent and much better than that shown by the turbidimetric method. By varying the normality of the solutions and the size and degree of accuracy of the burets, a wide range of micro quantities of selenium can be determined. Perhaps by improvements of the procedure lower quantities can be determined with lesser percentage

error than can be done today. However it must be recognized that there is possibility of reaching a definite lower range, below which it is impossible to use the volumetric method, except at a prohibitive sacrifice of accuracy. It is recommended that next year the Associate Referee determine this lower limit and devise another method, perhaps colorimetric, for still lower ranges.

ZINC

The Associate Referee on Zinc made no formal report. In a communication to the Referee he indicated (1) that the colorimetric resorcinol method was useless because the color produced was not proportional to the amount of zinc, (2) that he had not been very successful with the dithizone method, (3) that some attention had been given to Quick's borneol-glucuronic suggestion, and (4) that he had been trying to do something with a vacuum tube electrometric potassium permanganate titration procedure. In other words the Associate Referee has been laying the ground work for next year. In conclusion the Referee desires to call attention to three recent papers on the determination of zinc involving the use of dithizone: (1) The Determination of Zinc in Foods, *Analyst*, 1936, p. 734, by Sylvester and Hughes; (2) Nachweis und Bestimmung kleiner Zinkmengen mit Dithizone, by Hellmut Fischer und Grete Leopoldi, *Z. anal. Chem.*, **107**, 241 (1936); and (3) A Dithizone Method for the Measurement of Small Amounts of Zinc, by P. L. Hibbard, *Ind. Eng. Chem. Anal. Ed.*, **29**, 127 (1937). These papers do not show that dithizone methods for the determination of zinc have been perfected, but they do indicate a decided trend toward their use.

RECOMMENDATIONS[1]

It is recommended—

(1) That the results obtained this year with perchloric acid digestion and ashing with magnesium nitrate as a fixative in methods of sample preparation be confirmed.

(2) That arsine evolution as an isolation method for arsenic and not as a method of determination be studied next year.

(3) That work intended to extend the Gutzeit range be continued.

(4) That the study of arsenic extraction and the molybdenum blue method of arsenic determination be continued.

(5) That collaborative work on the carbamate copper method be continued for another year, with prospective tentative adoption in view, and that the Associate Referee undertake the study of photometric measurement of the color.

(6) That collaborative study of fluorine in phosphates and baking powder be continued, and that special attention be given next year to preparation of samples of organic material.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 60 (1937).

(7) That collaborative lead studies be initiated this year and that special attention be given to the study of lead methods for oils and baking powder and to the simplification of interference removal.

(8) That work on mercury determinations, especially photometric dithizone methods, be continued.

(9) That work on selenium be continued.

(10) That work on zinc be prosecuted with special vigor.

REPORT ON ARSENIC

By C. C. Cassil* (Bureau of Entomology and Plant Quarantine, Washington, D. C.)

The Gutzeit method, *Methods of Analysis, A.O.A.C.*, 1935, 370, has been subjected to some criticism. In the case of certain products that are difficult to oxidize, for example, tobacco and pine wood, it has been demonstrated that even though the arsenic is present in inorganic form certain unoxidized organic matter interferes with the evolution of arsine and causes low results. With other materials containing arsenic in organic combination, such as shrimp and cod liver oil, not only does the disturbance mentioned occur, but the arsenic is not completely converted to the inorganic form necessary for reduction to arsine. In all these cases a black color appears in the Gutzeit generator and indicates low results.

Gross[1] showed in the case of tobacco that the first type of interference could be overcome by isolating the arsenic from the digested solution as magnesium ammonium arsenate and then proceeding with the Gutzeit method. Remington and Coulson[2] and Carey *et al.*[3] presented a method involving the burning of the organic material with oxygen in an enclosed system. Both of these methods yield fairly accurate results, but they are long and require (especially the latter method) expert manipulation. A third method of destroying refractory materials has been suggested by Remington and Coulson,[2] who add 50 mg. of copper sulfate to the material being digested and boil for about 2 hours.

The writer found that copper sulfate does act as a catalyst to aid in breaking down refractory materials. Considerable interference was apparent when an aliquot part of the digested material was introduced into the Gutzeit generator and the potassium iodide reagent was added. A precipitate of cuprous iodide is formed, and the zinc is activated to give a different rate of hydrogen evolution. Remington and Coulson did not encounter this trouble because the material they used contained so much arsenic that they were able to dilute the solution to a comparatively

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* Appointed Associate Referee on Arsenic later.
[1] *Ind. Eng. Chem. Anal. Ed.*, 6, 327 (1934).
[2] *Ibid.*, 280.
[3] *Ibid.*, 327.

large volume and the aliquot part taken for analysis contained only a relatively small amount of copper.

The writer has also used mercuric oxide and selenium as catalysts. The former appears to break up the refractory material when it is submitted to prolonged boiling with sulfuric acid, but when the digested solution is introduced into the Gutzeit bottle the mercury forms a surface film on the zinc which renders it inert to the acid, and no hydrogen is evolved. Selenium seems to be an excellent catalyst but the same difficulty is encountered as in the case of copper.

PERCHLORIC ACID TREATMENT

Perchloric acid appears to be the solution to the problem of destroying interfering substances during the process of digestion and completely changing organic arsenic into the inorganic state. Kahane[1] has used this reagent for digestion purposes.

The writer has used the perchloric acid treatment with several organic substances known to be resistant to the ordinary sulfuric-nitric acid wet digestion, such as shrimp, tobacco, and cod liver oil. It was found that this treatment not only destroys the organic material completely but it materially shortens the time of digestion. Also smaller quantities of the other digestion reagents are required. For example, 20 cc. of sulfuric acid, 35 cc. of nitric acid, and 10 cc. of perchloric acid (60 per cent) are sufficient to completely digest 10 grams of any of the substances named. Furthermore, after the digestion is completed, no preliminary isolation treatment for these products is necessary previous to introducing the aliquot part into the Gutzeit bottle. The necessity of adding ammonium oxalate to the digested solution to destroy the nitrogen oxides, which interfere with the production of arsine, is also removed with this procedure because the perchloric acid breaks up the nitrosyl-sulfuric acid, and on further boiling most of the perchloric acid is evaporated, leaving the arsenic in the sulfuric acid as arsenic acid. If a small quantity of perchloric acid remains in the digested solution, it will not interfere in the Gutzeit generator but will act merely as acid to aid in the development of hydrogen. As much as 1.5 cc. of 60 per cent perchloric acid (in place of HCl) introduced directly into the generator did not alter the stain in any way.

The digestion is carried out as follows: the organic material is placed in the Kjeldahl flask; if the material is dry, a little water is added to prevent spontaneous combustion, and then a mixture of 20 cc. of sulfuric acid and 15 cc. of nitric acid is introduced. As soon as the initial reaction has subsided, heat is applied and more nitric acid is added in small portions from time to time as the material begins to turn brown or darken. After the second addition of nitric acid, 10 cc. of perchloric acid is added to the solution, and heating is continued. Usually the solution clears or turns

[1] *Comp. rend.*, 195, 48 (1932).

yellow (due to the liberation of chlorine) very shortly, but if carbonization occurs after the addition of perchloric acid a small portion of nitric acid will cause the solution to clear. Materials that contain refractory substances can be destroyed completely by prolonged boiling for 30–60 minutes, depending on the amount of such material present. The only absolute criterion for judging the required time for boiling is to run the Gutzeit analysis to see if the solution remains clear and does not turn black.

The perchloric acid must not be added too soon because there is danger of an explosion if a large quantity of organic material is present. Even with organic material that apparently does not react violently with perchloric acid, proper precautions should be taken. The greatest trouble in this respect was encountered with tobacco and shrimp; in the case of leaves and bees no trouble was experienced when the perchloric acid was added after the first charring of the substance.

When aliquot parts of these digested solutions were analyzed by the Gutzeit method, no interference was evident, and the results are the same as those obtained by the Remington "torch" method, and in the case of tobacco by the magnesium ammonium arsenate isolation. The reagent blank for the small amount of acid used was found to be negligible. This method of sample preparation is as satisfactory as any previously suggested for refractory materials and is much faster. Table 1 shows the results obtained on samples of shrimp and tobacco, by four methods.

TABLE 1.—*Results obtained with shrimp and tobacco by four methods of sample preparation*

METHOD OF SAMPLE PREPARATION	SHRIMP	TOBACCO
	mg. As_2O_3 in 10 gram sample	
Digestion with H_2SO_4 and HNO_3	0.090	0.050
	0.096	0.060
Digestion with H_2SO_4 and HNO_3, followed by phosphate precipitation	0.150	0.160
	0.145	0.150
Ashed in inclosed torch	0.204	0.159
	0.206	0.147
Perchloric acid treatment	0.210	0.165
	0.220	0.156

DRY ASHING

Cerium nitrate and magnesium nitrate were tested as ash-aids in this part of the investigation. The following procedure was used with shrimp and tobacco: Ten grams of sample, 2.5 grams of ash-aid, and 25 cc. of

nitric acid were thoroughly mixed and evaporated to dryness on the steam bath. The residue was then placed in a muffle at 500°C. for 2 hours. Visible carbon was present after this treatment, therefore the sample was moistened with nitric acid, again evaporated to dryness, and re-ashed

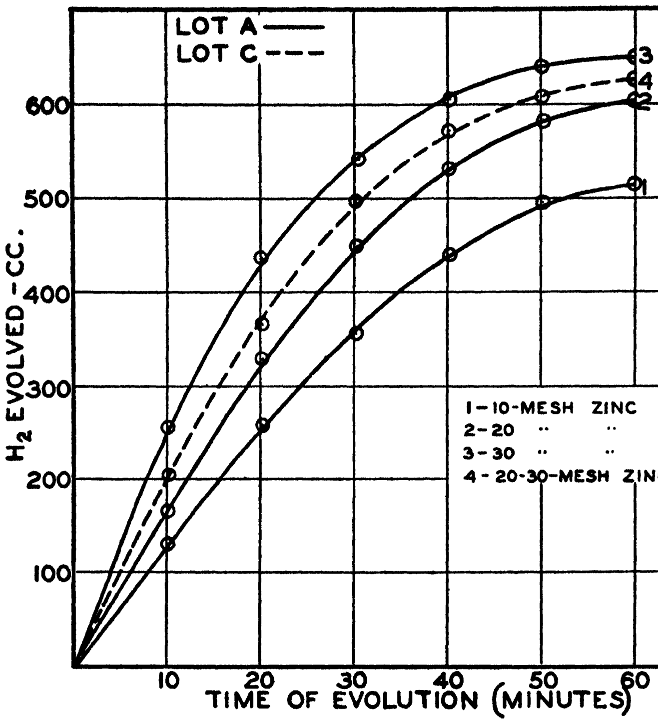

FIG. 1. SPHERICAL GRANULAR ZINC

for an hour at 500°C. This treatment produced a white ash, which was subsequently taken up in hydrochloric acid (1+3) and made to volume. Aliquot parts were taken for the Gutzeit analysis. The results are shown in Table 2.

TABLE 2.—*Results obtained by using different ash-aids*

ASH-AID	SHRIMP	TOBACCO
	mg. As_2O_3 in 10 gram sample	
$Mg(NO_3)_2$	0.210	0.150
$Ce(NO_3)_2$	0.035	0.015

With the cerium nitrate only 14 per cent of the arsenic was recovered in the case of the tobacco and 17 per cent from shrimp, but when mag-

nesium nitrate was used approximately 100 per cent recoveries of arsenic were obtained in both cases. The latter method offers an apparently satisfactory ashing procedure for analyzing materials for arsenic, but its use is not advised when the wet digestion process can be followed, because of the saving in time.

FINENESS OF ZINC VS. RATE OF HYDROGEN EVOLUTION

Of the several factors that control the length and intensity of the stain obtained with a given quantity of arsenic, the two principal ones

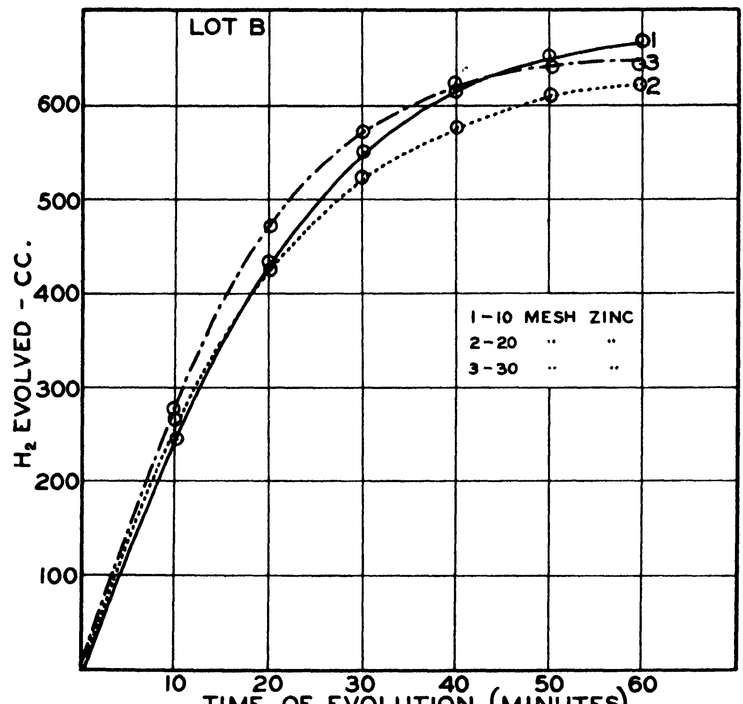

FIG. 2 MOSSY GRANULAR ZINC

are rate of hydrogen evolution and method of impregnating the strip. Many of the conditions controlling the arsine evolution, such as temperature and concentration of acid, may be kept constant in most laboratories, but the type of zinc used may vary greatly from bottle to bottle. It was therefore thought advisable to investigate the variations introduced by several types and particle sizes of zinc.

Three different grades of granular zinc, sold as 10-, 20-, and 30-mesh, were purchased from each of three sources (A, B, and D) and a tenth sample, designated 20–30-mesh, was obtained from a fourth company, C.

All of these samples were sifted and found to contain considerable amounts of dust. Those portions passing through a 40-mesh sieve were discarded. Each sample was then tested in duplicate for rate of hydrogen evolution, all conditions being the same as those described in the official Gutzeit method. The ten samples represented two distinct types of zinc particles, not only noticeable by texture but also by shape. These types will be referred to as "spherical granular," illustrated in Fig. 3, and "mossy granular," shown in Fig. 4.

The rates of hydrogen evolution produced by the three samples from Lot A and the one from Lot C, all of which were of the spherical granular

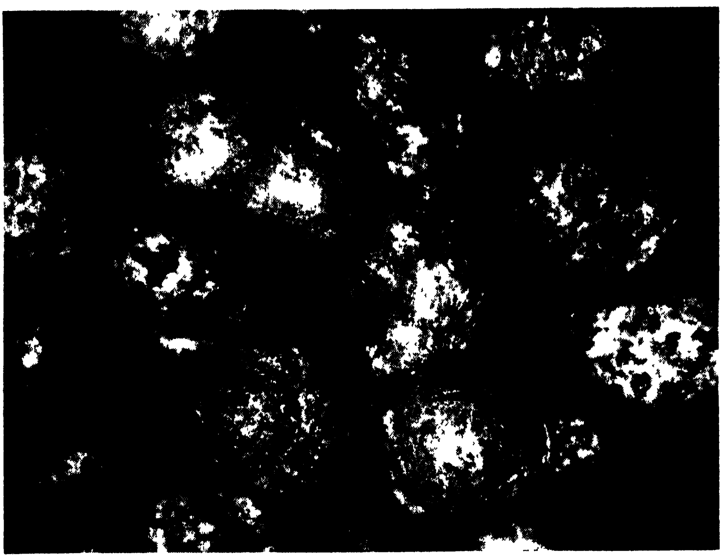

FIG. 3. SPHERICAL GRANULAR ZINC ×20

type (Fig. 1), differed in the order of their particle size, which is closely related to surface area, the 30-mesh being the most active and the 10-mesh the least. One sample from Lot D was of the same type and behaved similarly. The other five samples were of the mossy granular type and acted differently as shown by the three samples of Lot B in Fig. 2. There was no marked correlation between particle size and rate of evolution, their rate of evolution being approximately the same for the three sizes and about equal to that of the 30-mesh grade of the other type. It is obvious that a definite rate of hydrogen evolution cannot be assured by the use of a particular size of a mossy granular zinc. The most satisfactory stains were produced by 20-mesh spherical granular zinc.

IMPREGNATION OF STRIPS

The official method does not specify any definite procedure for impregnating the Gutzeit strips with mercuric bromide, concentration and time being left to the discretion of the analyst. In the past, this laboratory has obtained standard curves (mg. As_2O_3 plotted against mm. stain) whose slopes vary as much as 100 per cent, depending on how the strips have been impregnated and on the rate of arsine evolution. Several attempts have been made to overcome this variation, such as using different concentrations of mercuric bromide and varying the time for impregnating the strips, but none has produced the desired result.

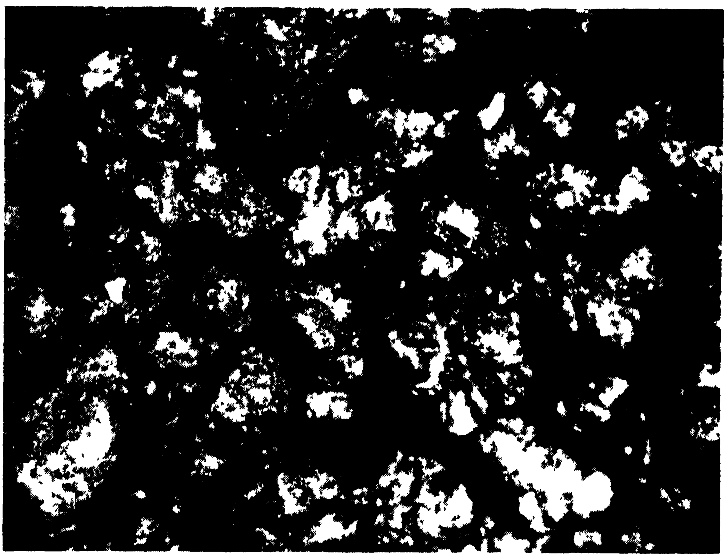

FIG 4. MOSSY GRANULAR ZINC ×20

A different method of impregnating the strips used recently in this laboratory produces standard curves that are of more nearly equal slope and in addition have less curvature. The strips are placed in a 3.5 per cent alcoholic solution of mercuric bromide and the container is then evacuated (70 mm. pressure) for a period of one hour, at which time the strips are removed and dried in the usual manner. This procedure pulls the air out of the paper strips, and more nearly uniform impregnation can be obtained. The optimum conditions have not been worked out in respect to concentration of the mercuric bromide and time of impregnation, but when they are it is believed that the standard curves will be more nearly uniform than they have been in the past. In addition, when

the strips are treated in this way, very sharp end points of the stains are obtained.

RECOMMENDATIONS

It is recommended—

(1) That cooperative work be done on checking the perchloric acid treatment and dry ashing for the preparation of samples.

(2) That optimum conditions be determined for the impregnation of the strips.

(3) That other colorimetric methods be investigated to find a more sensitive and accurate method than the Gutzeit.

(4) That a careful check be made on extending the range of the Gutzeit method.

REPORT ON COPPER

By E. J. Coulson (U. S. Bureau of Fisheries, Charleston, S. C.),* *Associate Referee*

This year further studies were conducted on colorimetric methods for the determination of copper in foods, with special attention given to the sodium diethyldithiocarbamate reagent. Inasmuch as the hydrogen sulfide methods described last year, *This Journal,* **19,** 219 (1936), for the separation of copper from interfering substances were tedious, time-consuming, and productive of unsatisfactory results, the search of methods of eliminating these difficulties was also continued.

Last year Winter, the Referee on Plants, *This Journal,* **19,** 360 (1936), proposed a modification of the Haddock-Evers method[1] for the determination of copper in plant materials that utilized the carbamate reagent. Iron interference and calcium phosphate precipitation were overcome by buffering the solution with citric acid, and the copper salt was shaken out with carbon tetrachloride. This method seemed to present such an advantage over the hydrogen sulfide methods that the associate referee decided to study it.

EFFECT OF OTHER METALS

In order to study the effect of other metallic elements on the copper determination, salts of various metals were added in varying concentrations to solutions with known quantities of copper and to copper-free solutions. Hydrochloric acid and citric acid were added, and the solutions were made slightly alkaline to litmus with ammonium hydroxide. The carbamate reagent was then added, and the solutions were extracted with measured quantities of carbon tetrachloride.

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* Present address, Bureau of Chemistry and Soils, Washington, D. C.

[1] *Analyst,* **57,** 495 (1932)

Lead, zinc, and mercury form white precipitates with the carbamate reagent in the ammoniacal solution, but these precipitates dissolve in carbon tetrachloride and the resultant solutions are colorless. Quantities of lead up to 8 mg. and zinc up to 16 mg. do not interfere in the copper estimation. Mercury produces a very insoluble compound with the carbamate reagent. When 4 mg. or more of mercury is present, it precipitates all the carbamate salt, but when an excess of the reagent is added the copper color appears.

Stannous tin above 1 mg. causes the carbon tetrachloride layer to be cloudy, but it can be centrifuged or filtered clear. The color is not affected. Stannic tin in quantities up to 8 mg. does not interfere.

Cadmium, when present in large quantities, produces a white precipitate with the carbamate reagent. This precipitate is insoluble in the carbon tetrachloride and produces cloudiness. The carbon tetrachloride can be centrifuged or filtered clear, however, and the copper color remains unaffected.

Arsenic and antimony produce no precipitates or color with the carbamate reagent, and quantities up to 8 mg. do not interfere.

Bismuth produces a yellowish green precipitate with the carbamate reagent in the ammoniacal solution and dissolves in the carbon tetrachloride, giving a pale yellow color instead of the golden brown color of the copper salt. In the presence of 0.02 mg. of copper quantities of bismuth up to about 0.1 mg. do not produce a tint that can be detected in the colorimeter when an artificial light and a blue filter are used, but the tint or off color can be detected by daylight. No method for overcoming the interference of bismuth was found, and this problem was postponed for the present.

Manganese (ous), if present in more than traces, produces a pink color in the carbon tetrachloride layer. It was found, however, that this color completely fades, leaving the copper color unaffected, if the tightly stoppered tube containing the carbon tetrachloride solution is allowed to stand overnight in a warm place. The fading does not take place if the solution is kept in the refrigerator. It was also found that sodium pyrophosphate is quite effective in preventing the interference of manganese. The pyrophosphate (5.0 cc. of a 4 per cent solution) is added after the solutions are made alkaline with ammonium hydroxide.

Ferrous iron, in quantities greater than 1 mg., produces a brown color in the carbon tetrachloride layer. At *p*H 8.0 ferric iron will also produce a brown color in the carbon tetrachloride layer. This interference can be effectively overcome, however, if the *p*H of the ammonium citrate solution is adjusted to about 8.5 before being shaken with carbon tetrachloride. This condition may be attained by adding concentrated ammonium hydroxide to the citric acid solution until just alkaline to litmus (blue) and then adding 0.2 cc. in excess. Under these conditions quantities

of ferric iron up to 10 mg. do not interfere. Haddock and Evers[1] state that copper can be accurately determined by this method in the presence of as much as 100 mg. of iron. According to Tompsett[2] the iron complex is not formed in the presence of pyrophosphate when the *p*H exceeds 7.5, or in the presence of citrates when the *p*H exceeds 9.0.

EFFECT OF *p*H

In the absence of large quantities of phosphates, moderate variations in the *p*H of the solutions apparently do not seriously affect the quantitative extraction of the copper carbamate salt. In Table 1 is shown the variation of the colorimeter readings with changes in *p*H. In this experiment each determination contained 1.5 grams of citric acid, 1 cc. of concentrated hydrochloric acid, and 0.02 mg. of copper. Concentrated ammonium hydroxide was added until the solutions were just alkaline to litmus (*p*H 8.0). Excess ammonium hydroxide in the quantities indicated and 10 cc. of the carbamate reagent were then added, and the volume of each was made up to 55 cc. The copper salt was shaken out in 5.0 cc. of carbon tetrachloride. The solution, which had been brought to *p*H 8.0 before extraction, was placed in the right-hand cup of the colorimeter and read against the other solutions, which were always placed at 20.0 mm.

TABLE 1.—*Effect of pH on extraction of copper*

EXCESS NH_4OH ADDED	*p*H	COLORIMETER READING*
Slightly acid	5.7	20.2
None	8.0	20.0
0.1 cc.	8.1	20.0
0.2 cc.	8.5	19.9
0.4 cc.	8.8	19.4
0.8 cc.	9.2	19.6

* Average of 5 readings

The results (Table 1) indicate that moderate excesses of ammonium hydroxide do not greatly increase the *p*H of the solutions and, furthermore, from *p*H 5.7 to *p*H 8.5 the color extracted is the same, while above *p*H 8.5 there is a very slight, but not a continued, decrease in color.

McFarlane[3] has shown that when dissolved in amyl alcohol the copper carbamate complex follows Beer's law. Haddock and Evers,[4] however, state that when compared in a Lovibond tintometer the color of this compound in carbon tetrachloride is not proportional to the concentration of copper when only the yellow constituent of the color is used. Winter[5] states that in very dilute solutions the color is directly propor-

[1] *Loc. cit*
[2] *Analyst*, 61, 591 (1936).
[3] *Biochem. J.*, 26, 1022 (1932).
[4] *Loc. cit.*
[5] *Loc. cit.*

tional to the amount of copper present, but in concentrated solutions it is necessary to compare the readings with a standard curve obtained by using known quantities of copper.

Two collaborators (D. L. Drabkin and L. V. Taylor, Jr.) studied the effect of reading the unknown solutions against the standard when set at different depths. Drabkin also investigated the effect of different filters, and decided that the following filter was best suited for this color: Wratten Filter (Eastman Kodak Co.) No. 62, Hg green. A good size is a $\frac{3}{4}$ inch circle, mounted in B glass. It is placed in or above the eyepiece of the colorimeter. The filter transmits a total of 3.5 per cent of the incident light, and of the light transmitted maximum transmission is at $\lambda 530 m\mu$ (green).

TABLE 2.—*Color filter as an aid in the colorimetry of copper carbamate solutions*

INVESTIGATOR	EXTRACTING SOLVENT	COPPER IN STANDARD	COLORIMETER READING STANDARD	COLORIMETER READING UNKNOWN	COPPER IN UNKNOWN (CALCULATED)	REMARKS
		mg.				
D. L. Drabkin	CCl_4	.03009	10	11.6	.0259	Without filter
			20	23.4	.0257	
			10	11.5	.0261	With filter*
			20	23.0	.0261	
	CCl_4	.03009	10	8.3	.0362	Without filter
			20	15.3	.0393	
			10	7.8	.0386	With filter*
			20	15.6	.0386	
	Isoamyl alcohol	.06018	10	8.0	.0752	Without filter
			20	15.3	.0787	
			10	7.5	.0802	With filter*
			20	15.0	.0802	
L. V. Taylor, Jr.	CCl_4	.0305	15	10.9	.0419	Daylight glass filter
			25	17.3	.0440	
	CCl_4	.0305	14.0	10.0	.0427	Daylight glass filter
			22.2	15.0	.0451	

* Wratten Filter (Eastman Kodak Co.) No. 62. Hg green.

In Table 2 are shown the results obtained by Drabkin on the copper carbamate solutions when read in a colorimeter with and without the aid of the filter described. Taylor's results, also shown in this table, indicate that the daylight filters furnished with certain illuminators are

not suitable for this color. Unfortunately the value of the color filter for these solutions was not fully appreciated when the following collaborative work was done.

COLLABORATIVE WORK

Two solutions were prepared for collaborative study in the manner described last year.[1] Solution 1 contained the ash of dried skim milk and Solution 2 contained the ash of dried spinach. Both solutions were freed from copper by hydrogen sulfide precipitation, and a known amount was again added. There was also added to Solution 2 the following quantities of other metals (mg. per liter): zinc, 200; lead, 50; tin, 50; mercury, 10; antimony, 10; arsenic, 20; and cadmium, 10.

These prepared solutions were sent out to 14 collaborators, with the request that they determine copper by the following proposed method:

MICRO METHOD FOR COPPER

REAGENTS

Use water redistilled from glass in all cases where dilutions are specified. Rinse the separatory funnels with dilute HNO_3 and then with redistilled water.

(a) *Sulfuric acid.*—Concentrated.

(b) *Hydrochloric acid.*—Concentrated.

(c) *Ammonium hydroxide.*—Concentrated.

(d) *Carbon tetrachloride.*—C.P.

(e) *Citric acid.*—Dissolve 150 grams of pure $H_3C_6H_5O_7 \cdot H_2O$ in water and dilute to 1 liter.

(f) *Sodium diethyldithiocarbamate solution.*—Dissolve 1 gram of sodium diethyldithiocarbamate in water, filter, and dilute to 1 liter.

(g) *Standard copper solution.*—Place approximately 0.5 g. of Bureau of Standards copper on the balance pan and weigh accurately. Transfer to a 125 cc. Erlenmeyer flask and add 15 cc. of HNO_3 (1+4). Cover the flask with a watch-glass and allow the copper to dissolve at room temperature. Heat to facilitate solution of the remaining undissolved copper, cool, add 1 cc. of the H_2SO_4 and evaporate cautiously to SO_3 fumes. Cool, dilute, with 10–15 cc. of redistilled water, and again evaporate to SO_3 fumes. Dilute to 500 cc. Prepare a working standard by diluting 10 cc. of this strong standard to 1 liter and calculate the copper equivalent per cc.

PROCEDURE

Transfer an aliquot of the test solution containing 0.01–0.1 mg. of copper to a separatory funnel and add 1 cc. of HCl and 10 cc. of the citric acid solution. Drop in a small piece of litmus paper and add NH_4OH to the solution, dropwise, while giving the funnel a rotary motion to mix the liquid as each drop is added until the solution is just alkaline to litmus, and then add 0.2 cc. in excess. Add 10 cc. of the diethyldithiocarbamate reagent and make up to a volume of 55 cc. Finally add 5 cc. (or 10 cc.) of carbon tetrachloride, *accurately measured*, and shake vigorously for several minutes. Allow the layers to separate, dry the stem of the funnel with a cotton applicator, and draw off the carbon tetrachloride solution into a dry, stoppered test tube. Filter if necessary. Compare in a colorimeter with a standard copper solution prepared in an exactly similar manner.

Analysis of the solutions by the associate referee, following the pro-

[1] *Loc. cit.*

posed method, gave the following results (mg. Cu per liter): 1.74, 1.76, and 1.77 for Solution 1; and 8.42, 8.50, and 8.50 for Solution 2.

The results from the collaborators that replied are shown in Tables 3 and 4.

TABLE 3.—*Collaborative results by proposed method*
(Expressed as mg. Cu per liter)

COLLABORATOR	SOLUTION NO. 1[1]		SOLUTION NO. 2[2]	
	INDIVIDUAL VALUES	AVERAGE	INDIVIDUAL VALUES	AVERAGE
Lillian W. Conn and	1.81		8.43	
A. H. Johnson	1.81		8.44	
Baltimore, Md.	1.86	1.83	8.49	8.45
D. L. Drabkin	1.72			
Philadelphia, Pa.	1.31	1.52		
	1.58		8.86	
	1.50		8.81	
	1.41	1.50[3]	8.71	
			8.63	8.75
R. B. Dustman and	1.82		9.08	
W. C. Brown	1.83		9.04	
Morgantown, W. Va.	1.71	1.78	8.68	8.93
	1.56		9.00	
	1.56		9.08	
	1.57	1.56[4]	8.68	8.92[4]
B. R. Fudge	1.83		7.38	
Lake Alfred, Fla.	1.77		7.08	
	1.80		7.68	
	1.79	1.80	7.26	7.35
W. E. Krauss and	1.67			
R. G. Washburn	1.67			
Wooster, Ohio	1.71	1.68		
	1.85		8.06	
	1.88		8.06	
	1.87		7.85	
	1.91		8.06	
	1.80		8.12	
	1.94		7.70	
	1.94		8.01	
	1.84		8.01	
	1.87		8.17	
	1.83	1.87	8.12	8.02

[1] Contains 1.70 mg. Cu per liter.
[2] Contains 8.50 mg. Cu per liter.
[3] Isoamyl alcohol as extracting solvent.
[4] Standard varied from unknown by less than .005 mg. of Cu.

TABLE 3 (*Continued*)

COLLABORATOR	SOLUTION NO. 1[1]		SOLUTION NO. 2[2]	
	INDIVIDUAL VALUES	AVERAGE	INDIVIDUAL VALUES	AVERAGE
G. T. Lewis	1.74		8.00	
Emory University, Ga.	1.67		7.06	
	1.58		7.32	
	1.55	1.64	7.23	7.46
			7.69	
L. S. Palmer and	1.47		9.31	
J. W. Nelson	1.60		9.68	
St. Paul, Minn.	1.90		9.46	
	1.64		9.60	
	1.64		9.46	
	1.38		9.46	
	1.42	1.58		9.60
W. S. Ritchie and	1.78		9.69	
E. B. Holland	1.78	1.78	9.78	9.74
Amherst, Mass.				
Olive Sheets and	1.59		8.53	
Melle Ward	1.43		8.66	
State College, Miss.	1.52	1.51	8.59	8.59
	1.59		8.58	
	1.43		8.70	
	1.55	1.53	8.81	8.70
C. L. Smith	1.93		8.50	
Charleston, S. C.	1.95	1.94	8.42	8.46
L. V. Taylor, Jr. and	2.01		8.60	
3 Associates	2.01		8.56	
Maywood, Ill.	1.97	2.00	8.56	
			8.96	
			9.26	8.79
	2.00		9.18	
	2.04		9.18	
	2.01	2.02	9.22	
			8.87	
			8.59	
			8.38	8.57
	1.98		8.78	
	1.95	1.97	8.78	
			8.68	8.75
	2.03		8.85	
	2.03		8.75	
	2.03	2.03	8.72	8.77

TABLE 3 (*Continued*)

COLLABORATOR	SOLUTION NO. 1[1]		SOLUTION NO. 2[2]	
	INDIVIDUAL VALUES	AVERAGE	INDIVIDUAL VALUES	AVERAGE
R. W. Titus	1.82			
Marysville, Ohio	1.79		8.73	
	1.78	1.79	8.58	8.66
O. B. Winter and	1.90		8.50	
Miss Butler	1.75		8.59	
East Lansing, Mich.	1.85		8.50	
	1.92		8.34	
	1.88	1.86		8.48
	1.85		9.00	
	1.63		9.00	
	1.72		9.00	
	1.85		8.90	8.97[5]
	1.75	1.76[5]		

[5] Copper read from standard curve.

TABLE 4.—*Collaborative results by other methods*
(Expressed as mg. Cu per liter)

COLLABORATOR	SOLUTION NO. 1[1]		SOLUTION NO. 2[2]		METHOD
	INDIVIDUAL VALUES	AVERAGE	INDIVIDUAL VALUES	AVERAGE	
D. L. Drabkin			7.98		Gebhardt-Sommer
			8.08	8.03	H_2S precipitation
					and carbamate
					method[3]
W. E. Krauss and	1.66		9.41		Carbamate - copper separated from
R. G. Washburn	1.66	1.66	9.41		calcium phosphate
			9.41		
			9.41	9.41	and iron by ammonia precipitation
			9.32		
			9.32		
			9.40		
			9.35	9.35	
C. A. Elvehjem	1.778				Dithizone[3] method. Hg interfered
and M. O. Schultze	1.846		10.13		
	1.820		9.78		in Solution 2
	1.856	1.83	10.22	10.04	

[1] Contained 1.70 mg. Cu per liter.
[2] Contained 8.50 mg. Cu per liter.
[3] See last year's Referee report (*loc. cit.*).

COMMENTS OF COLLABORATORS

W. C. Brown.—I found that more accurate results could be obtained on known amounts of copper if the standard was maintained within 0.005 mg. of copper per liter of the unknown.

Lillian W. Conn.—The only difficulty encountered in carrying out the procedure was the cloudiness of the CCl_4 extract, which made it necessary to filter the solutions before comparing with the standard. It seems that there should be a more exact method of adjusting the reaction. Under the conditions stated, litmus paper changes color so slowly that too much NH_4OH may be added. Using a beaker and stirring with a glass rod would facilitate mixing of the liquids. There was a slight concentration of color on standing 18 hours.

D. L. Drabkin.—From the results that I obtained with Solution 1, in which phosphates precipitate, it appears that with material of this type the *p*H control must be more critical than has been indicated in the directions. Slight increases in the amount of NH_4OH used appeared to yield progressively lower results. When litmus paper is immersed in solution, as in the present work, its color reaction is most sluggish, and the color sought as an end point is deceptive. I suggest that two control tubes be used for comparison, one with a piece of litmus paper in a buffer solution of *p*H 8.0 (purplish), the other with litmus paper in a buffer of *p*H 9.2 (blue). Due to sluggishness of reaction sufficient time must be allowed for color development.

I consider that amyl alcohol is preferable to CCl_4 because (1) it is less volatile and (2) it obviates the use of separatory funnels. Although separatory funnels may be used without greasing the stopcocks, unless the cocks are perfectly ground they leak and are hard to control. Grease may cause contamination, especially since a fat solvent is adjacent to the cock. In my experience, isoamyl alcohol has always quickly and readily separated from the solutions, even though they contained large amounts of precipitate. I have encountered some difficulty in obtaining clear extracts with CCl_4. In the case of Solution 1 emulsification occurred, making filtration necessary.

There is little evidence of fading (after 30 hours), but there is some evidence of concentration due to evaporation. The solutions were kept at room temperature in clean test tubes, stoppered with cork. It is my impression, based upon the results on two solutions only, that amyl alcohol extracts show no tendency to fade or concentrate when kept under similar conditions.

I strongly urge the adoption of a color filter for reading these solutions (see text).

B. R. Fudge.—I have found it necessary to grease the stopcocks before each run to prevent leaks, and since stopcock grease is readily soluble in CCl_4, forming a yellow solution, it is necessary to remove the excess that collects around the orifice in the stopcock with CCl_4. The cocks are kept open while being washed with CCl_4. The small amount of grease exposed to the CCl_4 when the cocks are closed is negligible. My results indicate that the color intensity diminishes very slightly when the Solution stands 24 hours.

W. E. Krauss and R. G. Washburn.—Our reaction to the proposed procedure is that it is very simple and easy of manipulation. The fact that all the operations are carried on in one piece of apparatus makes the possibility ot mechanical loss remote. No difficulty was encountered in getting close checks on repeated determinations, and should the reported values be close to the correct ones, we would be enthusiastic about the method. You will note, however, that our recoveries of added amounts of copper were poor.

G. T. Lewis.—On standing, the solutions of CCl_4 for some reason invariably became cloudy. Colorimeter readings were slightly lower (Cu content higher) than on the previous day. This may have been due to the cloudiness or to evaporation

of a slight amount of the CCl_4. The solutions were stored during the 24 hour period in test tubes stoppered with cork stoppers. At any rate, no fading was detected.

L. S. Palmer and J. W. Nelson.—The method is easy to carry out but the results of replicate determinations are not so concordant as one would expect. No considerable fading of the Solution was noted on standing. Solution 1 became cloudy when alkaline, while Solution 2 remained clear.

Olive Sheets and Melle Ward.—We had difficulty at first in obtaining clear solutions, but when we filtered the CCl_4 we obtained fairly good checks. The filtrations were made rapidly and the test tubes stoppered at once. The standard faded perceptibly after standing 24 hours, but the unknown had faded little if any.

L. V. Taylor, Jr.—The variations noted in the results were due to readings at various depths on the colorimeter. These were more pronounced in Solution 2. The results on Solution 2 were rather erratic.

R. W. Titus.—No difficulties were encountered in carrying out these determinations. As to fading of the copper carbamate color in the CCl_4 solution, we found that the standard (containing 0.03 mg. of copper) faded to about 60% of its original color over a 24 hour period. Of two samples of No. 1 solution held for 24 hours, one gave approximately the same reading, while the other showed considerable fading. Of two samples of No. 2 solution held for 24 hours, both gave about the same readings when first placed in the colorimeter cups, but in a very short time the solutions became cloudy and consequently higher results followed. In this connection, it might be stated that no effort was made to protect the solutions from light. The method was followed exactly as outlined, 0.2 cc. of NH_4OH being added to both solutions. This, however, caused some precipitation of calcium phosphate in Solution No. 1. We found the shades of copper carbamate difficult to read by artificial light.

DISCUSSION

In most cases the reported results on Solution 2 are quite close to the actual quantity present. The average values range from 7.35 to 9.74, averaging 8.63 mg. of copper per liter. However, if the two low values and the two high results are disregarded, the remaining 79 per cent is found within the range of 8.02–8.92, averaging 8.65 mg. per liter and with a probable error of ± 0.045. The actual quantity of copper added to this solution was 8.50 mg. per liter. Therefore, the maximum error of the method on the spinach sample and in the presence of added zinc, lead, tin, mercury, arsenic, antimony, and cadmium was 14.6 per cent, but 79 per cent of the values was within 5.6 per cent of the actual value.

The results on Solution 1 varied from 1.50 to 2.03 mg. of copper per liter, and the values are quite evenly distributed over this range. The mean value is 1.76. The actual quantity of copper added to this solution was 1.70 mg. per liter. The maximum error on the milk sample, therefore, was 19.5 per cent.

The large quantities of calcium phosphates in milk samples present considerable difficulty in the analysis of this material. The use of citric acid effectively prevented the precipitation of phosphates in the spinach solution when this was made alkaline with ammonium hydroxide but some precipitation occurred in the milk sample and there is evidence that the resultant occlusion of copper was responsible for the low results. On

the other hand, unless ammonium hydroxide was added until the *p*H was at least 8.5 iron interference was encountered and this may explain the high values. It is evident that there is need for further study before this procedure can be made applicable to the analysis of milk. The use of smaller samples, an increase in the quantity of citric acid, or extraction with successive portions of solvent, as recommended by Haddock and Evers, may be the answer.

There is some question also as to the most suitable solvent for extracting the copper compound, and substitution of isoamyl alcohol for the carbon tetrachloride may be found advisable. This would make it possible to discard the separatory funnels. One collaborator was able to trace some interference to the contamination of the carbon tetrachloride extract by stopcock grease.

The adoption of a proper color filter for reading these solutions is also advised. The color of the copper carbamate compound in carbon tetrachloride appears to be quite permanent although a few of the collaborators reported some fading in the solutions after they had stood 24 hours.

In its present form the method appears to be applicable to the determination of copper in materials similar to spinach, but it does not give sufficiently concordant results on materials containing large amounts of calcium phosphates.

It is therefore recommended[1] that this method be further studied along the lines indicated in the discussion.

No report on zinc was given by the associate referee.

REPORT ON FLUORINE IN FOODS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

A sample of dicalcium phosphate was distributed for collaborative work on fluorine. No restrictions were made in regard to the analytical methods to be used by the collaborators, but one procedure requiring peroxidized titanium (colorimetric) and one using thorium nitrate (volumetric) were distributed to those collaborators that requested methods.

The sample was drawn from commercial stock. The major portion was passed four times through a flour sifter to insure uniform mixing. The efficacy of this mixing was checked by repeating the procedure on another part of the sample, to which a pinch of lampblack was added. A uniformly gray mixture, free from black specks, resulted.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 60 (1937).

TABLE 1.—*Collaborative results*

ANALYST NUMBER	FLUORINE FOUND (p.p.m.) HIGH	LOW	AVERAGE	GENERAL AVERAGE	METHOD USED
1	—	—	10.0	10.0	(a)
2	—	—	14.0	14.0	(a)
3	16.0	13.0	15.0	15.0	(b)
4	18.0	12.0	15.0	15.0	(b)
5	17.0	16.0	16.3	15.9	(a)
5	16.0	14.0	15.5		(b)
6	—	—	20.3	20.2	(a)
6	—	—	20.0		(b)
7	24.0	13.5	21.6	21.6	(a)
8	25.0	18.7	21.9	21.9	(a)
9	—	—	22.3	22.3	(a)
10	30.0	30.0	30.0	30.0	(a)
11	—	—	31.0	30.5	(a)
11	—	—	30.0		(b)
12	34.7	30.0	32.5	32.5	(a)
13	40.0	30.0	33.0	33.0	(a)
14	33.7	32.8	33.1	33.1	(b)
15	33.0	30.0	31.5	33.3	(a)
15	—	—	37.0		(b)
16	—	—	34.0	34.0	(a)
17	36.0	34.5	35.8	35.8	(a)
18	38.0	36.0	37.0	37.0	(b)
19	40.0	35.0	37.5		(a)
19	40.0	38.0	39.0	37.5	(b)
19	37.0	35.0	36.0		(c)
20	—	—	38.0	38.0	(a)
21	—	—	38.0	38.0	(b)
22	—	—	39.0	38.0	(a)
22	—	—	37.0		(b)
23	—	—	38.0	38.0	(a)
24	39.0	37.7	38.4	38.4	(b)
25	46.0	30.0	39.2	39.2	(a)
26	40.0	40.0	40.0	40.0	(a)
26	40.0	40.0	40.0		(b)
27	48.0	35.0	40.3	40.3	(a)
28	42.3	39.6	40.7	40.6*	(a)
28	41.4	39.6	40.5		(b)
29	45.0	30.0	37.5	41.3	(a)
29	—	—	45.0		(b)
30	45.0	37.5	41.3	41.3	(a)
31	—	—	44.0	44.0	(a)
32†	—	—	44.0	44.0	(c)
33	—	—	49.0	49.0	(a)
34	—	—	55.0	55.0	(a)
35	67.0	62.0	65.0	65.0	(a)

(a) Peroxidized titanium method.
(b) Volumetric thorium nitrate method.
(c) Collaborators' own methods.
* Results found by the Associate Referee.
† Reported that with the peroxidized titanium method no definite results could be obtained, the variations being from 50 to 90 p.p.m.

Reports were received from the following collaborators:

(*A*) *Industrial Concerns:* Aluminum Company of America, American Agricultural Chemical Company, Calumet Baking Powder Company, General Chemical Company, Jacques Manufacturing Company, Malinckrodt Chemical Works, Monsanto Chemical Company, Parke, Davis & Company, The E. L. Patch Company, Phosphate Products Corporation, Royal Baking Powder Company, Southern Mineral Products Corporation, Upjohn Company, Victor Chemical Works, Wilson & Company, Zemmer Company.

(*B*) *State Departments:* North Dakota Regulatory Department.

(*C*) *U. S. Food and Drug Administration Analysts:* E. M. Hoshall, Baltimore; R. L. Horst, Boston; L. W. Ferris, Buffalo; K. L. Milstead, Chicago; J. Carol and H. G. Underwood, Cincinnati; L. H. McRoberts, Denver; E. L. Ellis and A. H. Wells, Los Angeles; S. B. Falck, New Orleans; J. H. Loughrey and G. Kirsten, New York; M. M. Jackson and M. Tubis, Philadelphia; H. W. Gerritz, San Francisco; R. H. Johnson, Seattle, and the Associate Referee.

The results in Table 1 are listed in order of increasing magnitude of the general average. Where several results were reported by the same method only high, low, and average are given.

The first reaction to the results reported in Table 1 must be that the method or methods are rather unsatisfactory. The Associate Referee feels, however, that the variations are often caused by apparently innocent modifications made by analysts in the original procedures. Several analysts reporting "off" results were asked to repeat their work and to follow to the letter a detailed procedure submitted by the Associate Referee. Table 2 gives results of these re-checks.

TABLE 2.—*Comparison of original and check results*
Fluorine in p.p.m.

ANALYST NUMBER	ORIGINAL REPORT	RE-CHECK	METHOD USED
1	10.0	37.0	(a)
2	14.0	35–37	(a)
3	—	36.0	(a)
3	15.0	36.5	(b)
4	—	36.0	(a)
4	15.0	35.0	(b)
5	16.3	35–37	(a)

Five of these results (Table 2) were submitted before the results of other reporting analysts were made known to the collaborators. The other two determinations were made in the presence of the Associate Referee. In two cases (Analysts 9 and 35), where the original subdivision was used up in the first analysis, re-checks on a new subdivision gave 37 and 45 p.p.m., respectively.

In all these determinations the distillation seems to have been the chief source of error. Such factors as the amounts of perchloric acid to use, the temperature at which the distillation is made, and the amount of dis-

tillate collected *at that temperature* are of prime importance. A study of these and other factors and their influence on the recovery of fluorine in the distillation with sulfuric acid has already been reported by Dahle and Wichmann, *This Journal*, **19**, 313 (1936).

The substitution of perchloric for sulfuric acid, recommended in connection with calcium phosphates, introduces another factor, the perchloric acid itself. The Associate Referee has found several batches of this acid giving unduly high amounts of volatile acids on distillation, and its use invariably caused high results for fluorine, particularly when the volumetric method was used. The recent work of Armstrong[1] may shed some light on this source of error.

A study of distillation recoveries with perchloric acid and phosphoric acid has been made. The complete data are given on p. 297.

It is recommended[2] that the work on fluorine in foods be continued, and that methods of isolation and the evaluation of existing methods of determination be studied.

REPORT ON LEAD

By P. A. Clifford (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The general colorimetric dithizone method for the determination of lead described at last year's meeting, *This Journal*, **19**, 130, 214, 232 (1936), has been used with increasing confidence during the year and has been found to be especially effective in the determination of quantities of lead below 0.05 mg. The neutral wedge photometer or photometric colorimeter used has proved to be particularly effective with this procedure, and sixteen of these instruments have been built and installed in the laboratories of the Food and Drug Administration. They are also being applied to the determination of fluorine, indole, carotene, lactic acid, and arsenic. The instrument, which may soon be commercially available, will be fully described in a future publication.

Two of the most promising methods for the determination of lead, the electrolytic and dithizone, have been proposed as tentative procedures, and if collaborative study is successful it is expected that they will be made official.

Since the colorimetric dithizone procedure was written up for inclusion in the 1935 edition of *Methods of Analysis*, it has been found that more explicit directions for the preparation of the ammonia-cyanide mixture are necessary. The general procedure calls for isolation of the lead in 50 cc. of 1 per cent nitric acid. This acid is then neutralized, and the mixture is made ammoniacal with an ammonia-cyanide mixture of specified strength

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[1] *Ind. Eng. Chem. Anal. Ed.*, **8**, 384 (1936).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 60 (1937).

in order to produce the optimum conditions for the subsequent color development. Either potassium or sodium cyanide is used in the preparation of the ammonia-cyanide reagent, and after neutralization of the acid the resultant *p*H is about 9.6–9.7 in either case. However, it has been found that traces of phosphate, present as an impurity in the cyanide, will precipitate traces of lead at this point and render the color development incomplete. This precipitation is usually slow, and if the color development is made immediately after the addition of the ammonia-cyanide reagent to the 1 per cent acid, the effect, in the case of potassium cyanide, is usually negligible. However, if an appreciable length of time (3 to 5 minutes) is allowed to lapse between the addition of the ammonia-potassium cyanide reagent and the color development, a significant loss of lead is usually observed.

With sodium cyanide, which usually contains more phosphate as impurity, this precipitation effect is even more pronounced, and batches have been found which were entirely unsuitable for this purpose.

To prevent the precipitation of lead phosphate by the use of citric acid and to purify the cyanide used are two ways out of the difficulty. The latter alternative is preferred by the Associate Referee because the use of citric acid complicates the preparation of the reagent in that the citric acid must itself be purified from traces of lead, and any method of purification involves partial or complete neutralization with ammonia. This complicates the *p*H adjustment before the color development. Further, unless large quantities are used, citric acid may not be completely effective in preventing the precipitation of lead phosphate.

Both potassium and sodium cyanide may be purified from traces of phosphate by recrystallization from aqueous solution. The procedure is to boil down a strong solution until precipitation begins, then to cool under the tap and filter off the crystals on a Büchner funnel. With potassium cyanide the filtrate usually may be further concentrated by boiling and a second crop obtained. However, this may not be advisable with sodium cyanide, as the second crop may contain enough phosphate to interfere, and it is sometimes necessary to recrystallize sodium cyanide twice to obtain a satisfactory product. The filtered crystals may be dried in vacuo at room temperature, but it is better, in order to avoid conversion to carbonate, to dissolve the moist crystals without drying them, and to adjust the solution to 10 per cent W/V strength by a specific gravity determination. For this purpose take the specific gravity $\left(\frac{20^\circ}{20^\circ}\right)$ of a 10 per cent W/V solution of ordinary reagent grade potassium cyanide as 1.0470 and of a 10 per cent solution of sodium cyanide as 1.0500. Concentrations of potassium cyanide or sodium cyanide solutions in the range 10–30 per cent may be determined quite accurately by means of

the following formulas, and thus permit dilution down to the prescribed 10 per cent reagent strength:

$$\%\ NaCN = \frac{\text{sp. gr.} - 1.0052}{0.00454}$$

$$\%\ KCN = \frac{\text{sp. gr.} - 1.0028}{0.00444}$$

In any case, check the suitability of the cyanide reagent as follows:

Prepare two standards containing identical amounts of lead. In one case, add the ammonia-cyanide mixture and develop the color immediately. In the other, allow 15 minutes to elapse between the addition of the ammonia-cyanide reagent and the color development. If the cyanide is sufficiently free of phosphate, the color difference between the two standards will not be appreciable, regardless of the method of color measurement. If the second standard indicates a lower amount of lead, some precipitation has occurred, and the cyanide is in need of further purification.

If photometric methods are used, the standardization curve made by plotting the amount of lead against the absorption coefficient ($-\log T$) should always be a perfectly straight line.

Because of the difficulty in obtaining pure sodium cyanide, the use of potassium cyanide is favored in the preparation of the ammonia-cyanide reagent. The advantage of sodium cyanide is in the preliminary extraction of lead where perchloric acid is used in treating the ash, as sodium perchlorate is more soluble than the corresponding potassium salt. Phosphate in the sodium cyanide should not interfere in preliminary extractions because they are always conducted in the presence of citrate, usually at lower *p*H, and always with a considerable amount of excess dithizone.

In working with samples of foliage during the past year, difficulty was experienced in filtering the hydrochloric acid solution of the ash, before isolation of the lead, due to the presence of quite large amounts of silica. It was found very helpful, after the ash had been dissolved in hydrochloric acid and the solution brought to dryness, to dehydrate the silica by fuming with 5 cc. of strong perchloric acid. After being taken up with water, filtrations of the ash solutions were entirely satisfactory. This expedient seems worthy of note. However, if the quantity of dehydrated silica is seen to be large it becomes necessary to apply a further treatment for the release of occluded lead (as much as 10 per cent of the total lead may be occluded in certain cases). To do so rinse the silica from the filter into a platinum dish, evaporate to dryness, and treat with one or two 5 cc. portions of hydrofluoric acid. Evaporate to dryness, take up with either hot hydrochloric or perchloric acid and add to the bulk of the ash filtrate.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be undertaken.

(2) That the directions for Reagents **13(j)** and **13(p)** in the 1935 Edition of *Methods of Analysis*, pp. 378–79, be amended by the addition of the word "phosphate-free" before the word "KCN."

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 60 (1937).

(3) That work be continued on special products, notably oils and fats, which have given difficulty with the regular methods of analysis.

No report on mercury was given by the associate referee.

REPORT ON SELENIUM

By J. A. Mathews, A. L. Curl, and R. A. Osborn, *Associate Referee*
(U. S. Food and Drug Administration, Washington, D. C.)

In last year's report, *This Journal*, **19**, 236 (1936), reference was made to the work of others, and proposals were made for further study. For various reasons not all of the procedures mentioned could be subjected to critical examination. A more accurate method of determining selenium was considered to be necessary before such comparisons could profitably be made, and the greater portion of the year's work was devoted to this problem.

Procedures of sample preparation, isolation of selenium, and the determinations used will be described and discussed briefly.

SAMPLE PREPARATION

A comparison of a wet digestion with an alkaline ashing procedure of sample preparation led to the conclusion that the former is preferable, at least for samples of organic material that contain relatively large quantities of selenium. For example, five determinations with a standard sample of vegetation treated by the wet digestion procedure followed by isolation and volumetric determination averaged 155 p.p.m. (138–166 p.p.m.) of selenium, while five determinations with alkaline ashing followed by an identical procedure of isolation and volumetric determination averaged 94 p.p.m. (88–99 p.p.m.). It is observed that losses of approximately 40 per cent were obtained by the alkaline ashing procedure. An unsuccessful attempt was made to apply the procedure described in *Methods of Analysis*, 1935, 321, **12**, for the determination of total sulfur in fruit products, to the preparation of selenium samples. This procedure calls for digestion of the sample with nitric acid and magnesium oxide or nitrate followed by ashing at 525°C. in a muffle furnace. Losses of selenium up to 100 per cent may occur. A substitution of other fixatives for magnesium, such as calcium, aluminum, and barium, was without benefit. While ashing of selenium samples under acidic conditions is definitely unsatisfactory, experiments with alkaline ashing and a fixative indicate that the selenium is not so readily lost. By modifying the conditions it may be possible to ash the samples without material selenium losses.

WET DIGESTION

The wet digestion procedure was modified somewhat from that described by Williams and Lakin.[1] A complete digestion of the sample in a system having a condenser and trap is substituted for incomplete digestion in an open beaker at a regulated temperature. At this time, however, no data are available to indicate a superiority of this procedure over that of Williams and Lakin. Variations in results from the turbidimetric method of determination were greater than the differences indicated by these modifications in sample preparation. With the development of the more accurate volumetric procedure of determination, it may be possible to compare these two procedures more critically.

DETERMINATION

Studies were made on different methods of determination, including the turbidimetric, volumetric, gravimetric, and colorimetric. Attempts were made to develop satisfactory procedures and to determine the quantities of selenium that can be so measured, as well as the accuracy and precision of the measurements.

Turbidimetric Procedure

The results shown (Table 1) were obtained by one of the writers when following the turbidimetric procedure described.

The quantities of selenium shown in the first column were given as unknowns, and the turbidities were matched against standards. It will be observed that it was possible to detect as small an amount of selenium as 1.5 micrograms (gammas), and that over the range 1.5 micrograms to 2 mg. approximately the same average deviation occurred, which is about 50 per cent of the maximum deviation.

By reducing the quantities of reagents, a still smaller amount of selenium than that shown in the table can be measured turbidimetrically. It was found, for example, that by a modified procedure it was possible to measure 0.4–2.0 gammas with no greater error than 0.2 gamma. The selenium was dissolved from the pad with 0.5 cc. of the acid and bromine mixture, the solution and 0.5 cc. of washings were collected in a 3-inch test tube having a capacity of 3 cc.; and the turbidity was developed in a total volume of 2 cc., as described for the larger amounts, and matched against standards containing 0.4, 0.7, 1.0, 1.5, and 2.0 gammas.

The hydrobromic acid used in turbidimetric work should be water white when treated with sulfur dioxide or sulfite. Recovered or redistilled acid may be used.

A standard selenium solution may be prepared from crude selenium by dissolving it in a mixture of hydrobromic acid and bromine followed by distillation and precipitation. The precipitate is filtered; washed

[1] Ind. Eng. Chem. Anal. Ed., 7, 409 (1935).

TABLE 1.—*Turbidimetric determination of selenium*
Range 2–15 micrograms (gammas) with standards of blank, 2, 4, 6, 8, 10, and 15 gammas

PRESENT	FOUND	DEVIATION	% DEVIATION
1.5	1.5	0	0
2	1.5	0.5	25
4	5	1	25
5	6	1	20
6	7	1	17
7	8	1	14
8	7	1	13
9	11	2	22
10	10	0	0
			Av. dev. 15%
Range 15–200 micrograms (gammas) with standards of 15, 20, 30, 45, 70, 100, 150, and 200 gammas			
13	13	0	0
18	19	1	6
22	20	2	9
25	18	7	28
30	25	5	17
50	45	5	10
80	70	10	13
100	125	25	25
130	175	45	35
160	150	10	6
			Av. dev. 15%
Range 200–2000 micrograms (gammas) with standards of 200, 300, 400, 500, 700, 1000, 1500, and 2000 gammas			
200	180	20	10
300	300	0	0
450	400	50	11
600	400	200	33
740	500	240	32
1000	1000	0	0
1160	700	460	40
1460	1000	460	32
1700	1500	200	12
1780	1500	280	16
			Av. dev. 19%

thoroughly with water, 95 per cent alcohol, and ether; and dried at 100°C. to constant weight. One gram of the pure selenium is dissolved in hydrobromic acid and bromine in a 1 liter volumetric flask, all but a trace of the excess bromine being destroyed by addition of sulfur dioxide water; the remaining bromine is destroyed by addition of a few drops of 5 per cent phenol solution; and the solution is diluted to volume.

From this solution more dilute standard solutions may be prepared. It is advisable to check the standard solutions thus prepared by methods of determination described herein.

The use of gum arabic as a stabilizing agent, as described in *Methods of Analysis, A.O.A.C.*, 1935, 14, was found to be objectionable, as the gum itself produces a turbidity and limits the accuracy of measurement, particularly in the lower ranges. Prolonged heating of the gum in acid solution will produce a color that will also interfere with the measurement.

Volumetric Determination of Selenium

The iodine and thiosulfate solutions should be standardized against a standard selenium solution or against a recognized ultimate standard. Thiosulfate can be stabilized by rendering it slightly alkaline with 2 drops of 50 per cent sodium hydroxide per liter. A standard selenium solution containing 0.1 mg. per cc. can be conveniently used to standardize 0.01 *N* thiosulfate and iodine. More dilute iodine and thiosulfate solution can be prepared as needed by dilution of standard 0.1 *N* solutions.

With 10 ml. burets 0.01 *N* iodine and thiosulfate solutions can be used to measure amounts of selenium from 100 to 2000 micrograms (gammas); 1 ml. microburets are suitable for the range 10–200 gammas. With 10 ml. burets and 0.001 *N* iodine and thiosulfate, the range 10–200 gammas can be covered. With the same solutions in 1 ml. burets, 1–20 gammas can be measured (0.0001 *N* iodine and thiosulfate in the 10 ml. buret for the range 1–20 gammas of selenium offered no advantage over the 0.001 *N* solutions in the smaller burets).

The apparatus for titration (see picture) consists of a stirring device, and calomel and platinum electrodes connected to a direct current amplifier with a sensitive galvanometer as a null indicator. The speed of stirring should be adjustable. The calomel electrode is preferably of the dipping type with ground-glass sleeve, and the platinum electrode is of No. 18 wire sealed into glass with about ¼" exposed. The circuit of the amplifier is shown in the accompanying sketch. The galvanometer is of the lamp and scale type with a sensitivity of 0.025 microamperes per scale division. Ground-glass extensions that dip under the solution to be titrated are attached to the tips of the burets. The tips of the extensions are constricted to permit emptying the buret in 4–5 minutes.

Table 2 indicates the type of results obtainable when the volumetric procedure and the titration apparatus described are used. Measured amounts of standard selenium solutions were titrated as unknowns. A comparison of these data with those obtained turbidimetrically indicates the greater accuracy and precision of the volumetric method.

It was found that one gamma of selenium could be distilled, precipitated, and measured volumetrically.

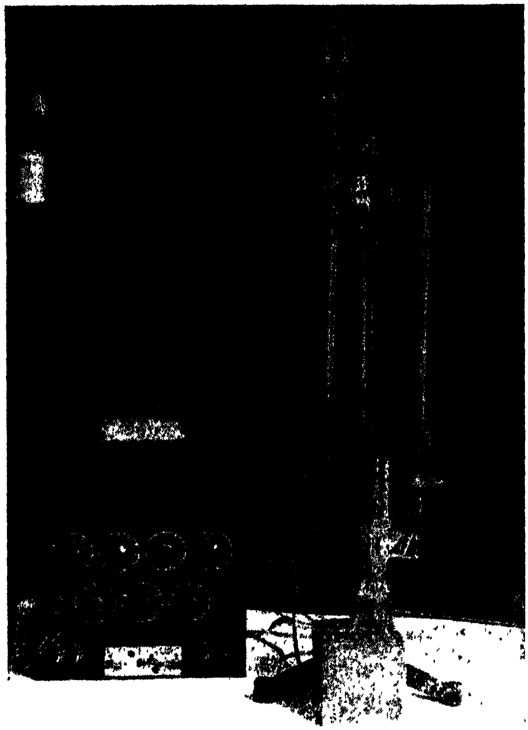

FIG. 1.—APPARATUS FOR THE ELECTROMETRIC TITRATION OF SELENIUM

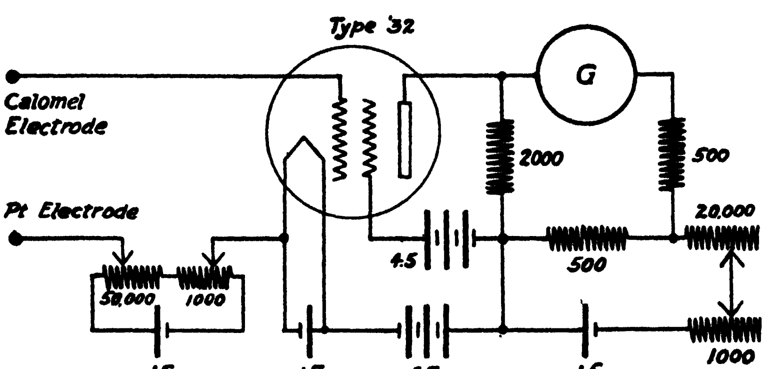

FIG. 2.—WIRING DIAGRAM OF DIRECT CURRENT AMPLIFIER AND GALVANOMETER CIRCUITS

TABLE 2.—*Volumetric determination of selenium (gammas)*

Se AS GIVEN IN UNKNOWNS	FOUND	DEVIATION	% DEVIATION	Se AS GIVEN IN UNKNOWNS	FOUND	DEVIATION	% DEVIATION
0.001 N thiosulfate and iodine in 1 ml. burets							
0.00	0.24	+0.24	—	4.00	4.02	+0.02	0.5
0.00	0.26	+0.26	—	4.60	4.68	+0.08	1.7
0.00	0.28	+0.28	—				
0.92	0.80	−0.12	13.0	5.92	5.66	−0.26	4.4
2.50	2.44	−0.06	2.4	6.00	5.68	−0.32	5.3
3.16	2.80	−0.36	11.4	7.68	7.58	−0.10	1.3
4.00	3.90	−0.10	2.5	9.86	10.12	+0.30	3.0
						Av. dev.	4.5%
0.001 N thiosulfate and iodine in 10 ml. burets							
0.00	+0.4	+0.4	—	60.0	60.2	+0.2	0.3
0.00	−0.8	−0.8	—	68.8	68.3	−0.5	0.7
0.00	+0.8	+0.8	—	80.0	80.8	+0.8	1.0
10.4	10.3	−0.1	1.0	98.2	98.0	−0.2	0.2
19.2	20.0	+0.8	4.2	120.0	119.7	−0.3	0.3
26.0	25.3	−0.7	2.7	128.2	128.5	+0.3	0.2
28.6	28.3	−0.3	1.0	160.0	158.2	−1.8	1.1
40.0	39.4	+0.6	1.5	197.2	196.3	−0.9	0.5
41.2	40.6	−0.6	1.5				
						Av. dev.	1.1%
0.01 *N* thiosulfate and iodine in 10 ml. burets							
0	0	0	—	419	420	+ 1	0.2
0	−2	− 2	—	508	503	− 5	1.0
85	82	− 3	3.5	574	590	+16	2.8
116	122	+ 6	5.2	661	661	0	0.0
142	139	− 3	2.1	788	800	+12	1.5
193	206	+13	6.7	995	994	− 1	0.1
231	226	− 5	2.2	1232	1240	+ 8	0.6
253	246	− 7	2.8	1502	1483	−19	1.3
320	317	− 3	0.9	1772	1772	0	0.0
403	400	− 3	0.7	1792	1788	- 4	0.2
						Av. dev.	1.8%

A study was made of a volumetric method of measuring selenium, in which selenious acid was reduced in acid solution with hydriodic acid to form free iodine and free selenium, the liberated iodine being titrated with standard thiosulfate. The conditions of acidity and the titration set up are essentially the same as those described previously. While no comparative data are tabulated, it was observed that of the two methods the reduction with excess thiosulfate is preferable, in spite of the fact that the visual indication of liberated iodine and selenium permits the analyst to estimate the approximate amount of standard solution necessary for the titration.

For amounts of selenium that are suitably titrated with 0.01 *N* standard solution there appears to be little choice between the two procedures. An objection to the reduction with iodine has been made by Gooch and Reynolds[1]; it is based on the adsorption of iodine by the precipitated selenium, which results in an indistinct and somewhat variable end point. Evidence of this type of adsorption was not apparent for amounts of selenium less than 1 mg.

The volumetric data contained in the tables represent a degree of precision that is believed to be better than has hitherto been reported. Under the conditions of titration one drop or less of any of the standard solutions produces an easily perceptible deflection of the galvanometer at the end point. It is believed that variation in reproducibility lies in the chemistry of the volumetric reaction rather than in the electrical set-up.

Colorimetric Determination of Selenium

Studies on a colorimetric method for measurement of selenium have not advanced to a stage where a finished procedure can be given. Such studies as have been made relate to a suitable method for dissolving selenium from the filter pad (after the preparation of the sample and isolation of selenium) and obtaining a solution of selenious acid in concentrated sulfuric acid that is free from other oxidizing impurities, and to the subsequent development of a relatively stable blue color upon addition of a few drops of 3 per cent codeine sulfate reagent.

It was found that selenium could be dissolved by placing the pad and selenium in a flask with a small amount of nitric acid, attaching a vertical condenser, and refluxing. The resulting solution can be filtered from the asbestos, and the filtrate and washings evaporated to dryness on a steam bath without loss of selenium. This fact has been demonstrated by reprecipitation of the selenium and volumetric determination. The selenium dioxide so obtained, however, still contains a trace of nitrate, which reacts with codeine reagent and interferes with the colorimetric measurement. It may yet be possible to remove these traces of nitrate by taking up in water and evaporating repeatedly or by baking out under carefully regulated conditions. A brief experiment, which will be continued, further suggests the possibility of dissolving the selenium from the pad with concentrated sulfuric acid containing a small amount of free bromine, followed by removal of the excess bromine with a few drops of strong phenol solution, and filtering if necessary before addition of the codeine reagent.

It was found that amounts of selenium from 1 to 100 gammas can be measured colorimetrically. Selenium standards containing blank, 1, 2, 5, 10, 25, 50, and 100 gammas of selenium in 10 cc. of sulfuric acid produced a well defined series of colors upon the addition of a few drops of codeine sulfate. Owing to a gradual fading of the colors, it was not possible to

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[1] *Am. J. Sci.* (3), 50, 254 (1895).

measure these standards satisfactorily by use of the Clifford neutral wedge photometer, Clifford and Wichmann, *This Journal*, 19, 130 (1936). Addition of a small quantity (0.1 mg. or less) of ferric chloride to the standards inhibited the fading effect but produced a small amount of color in the blank.

Gravimetric Determination of Selenium

The gravimetric procedure for selenium given in *Methods of Analysis, A. O. A. C.*, 1935, page 15, is suitable. If the analyst wishes, he may check the increase in weight of the Gooch crucible containing the selenium precipitate against the loss in weight obtained after volatilization of the selenium by heating for a sufficient period at 550°C.

The method formulated by the writers follows:

METHOD

PREPARATION OF SAMPLE

Place 10 grams of sample and 0.5 gram of HgO in a 500 cc. Kjeldahl flask having a 20 mm. interchangeable joint. Add a cold mixture of 50 cc. of H_2SO_4 and 100 cc of HNO_3 and mix thoroughly. Connect the flask to a modified extractor and the condenser units of a Soxhlet apparatus. (Modify the extractor unit by elimination of the siphon. Preferably construct a trap chamber similar to the middle unit of a Soxhlet without a siphon, with a 3 cm. vacuum unit immediately below the receiving chamber.) Heat very gently at the start. After the first reaction has subsided, heat gently until NO_2 fumes are no longer evolved freely, then heat more vigorously until all organic matter is destroyed. Cool, and if necessary (not necessary unless the highest accuracy is desired), slowly return the distillate to the digestion flask, which is kept as cool as possible. Again distil to SO_3 fumes and discard the distillate.

ISOLATION OF SELENIUM

Add 25 cc. of water to the cold H_2SO_4 digest, cool again, and add 50–60 cc. of HBr containing 0.5% by volume of free Br_2. Attach the flask to an all-glass distilling apparatus equipped with a thermometer and distil to 130°C., keeping the receiving flask cool. If the distillate contains insoluble material, filter through asbestos and wash. Saturate the filtrate with SO_2 gas, add 0.1 gram of $NH_2OH \cdot HCl$ and warm on a steam bath to 80°C. for 15 minutes. Allow to cool and filter through an asbestos gooch or Jena glass filter No. 4 and wash. (The filtrate may be saved for recovery of HBr.)

DETERMINATION

Turbidimetric procedure

Estimate the amount of selenium on the pad and dissolve with 10 cc. of HBr containing 1% by volume of free Br_2. Collect the filtrate and washings in a 25 ml. volumetric flask. Prepare selenium standards in duplicate for the range indicated by the estimation. (A good set of standards can be obtained by having each unit of the series 33–50% stronger than the next lower unit.) Add to each standard 10 cc. of $HBr+Br_2$. Nearly decolorize the samples and standards with a strong Na_2SO_3 solution, cool, and add 1 cc. of 10% $NH_2OH \cdot HCl$ solution. Dilute to 25 cc., mix, and warm on the steam bath to 80°C. for 30 minutes. Cool, and compare the samples and standards in a beam of light in a dark room. A satisfactory arrangement is to have a 60-watt lamp in a box painted dead black on the inside and outside, with a slit on the top about ½″ wide and 4–5″ long.

Volumetric Procedure

Dissolve the selenium on the pad with 2 cc. of HBr containing 1% by volume of free Br_2. Add the acid from a pipet, using a few drops to dissolve any selenium that may be left in the precipitation flask. Wash the flask and crucible with 5–10 cc. of water. Transfer the filtrate and washings to a 50 cc. beaker and dilute to 20 cc. Add SO_2 water dropwise until only a trace of free Br_2 remains. Gently blow over the top of the beaker to remove traces of SO_2 gas. Add dropwise sufficient 5% phenol (1–3 drops) to discharge the Br_2 color. Add a measured excess (not to exceed 10 cc.) of standard thiosulfate solution. Allow to stand several minutes and titrate electrometrically with standard I_2 of approximately equal strength. If the back titration is less than 1 cc., again add 5 cc. of standard thiosulfate solution and back titrate with standard I_2 solution for the end point.

RECOMMENDATIONS[1]

It is recommended that studies on the determination of selenium in foods be continued, with particular attention given to improvements in methods of sample preparation and colorimetric and volumetric procedures of determination.

REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

No report was received from the Associate Referee on Soluble Solids and Effect of Acids on Sugars on Drying. The Associate Referee on Pectic Acid and Electrometric Titration of Acidity submitted a preliminary report on electrometric titration. No collaborative work was done on inactive malic acid. Regarding the subject of lactic acid, it is believed advisable to defer further study of the method recommended last year, pending the outcome of the investigation of the colorimetric method that is being conducted by the Associate Referee on Dried Milk. The colorimetric method has been found satisfactory for milk products, and from all indications it should be applicable to fruit products and foods generally. It is not only simpler than the proposed method, but it is also less time-consuming.

It is recommended[2]—

(1) That last year's recommendations on soluble solids and effect of acids on sugar on drying, pectic acid and electrometric titration of acidity, and inactive malic acid be carried over for further study.

(2) That the colorimetric method for the determination of lactic acid reported at this meeting by the Associate Referee on Dried Milk be tried on fruits and fruit products.

No report on soluble solids and effect of acids on sugar on drying was given by the associate referee.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 60 (1937).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 65 (1937).

REPORT ON ELECTROMETRIC TITRATION OF ACIDITY

By R. U. BONNAR (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

As a result of work on this subject done up to the present time, a preliminary estimate is available as to the requirements in technic for the classical electrometric titration.

Table 1 shows a plot of the titration of a tartaric acid solution compared with that for a wine. Both are calculated to a common scale. It is easy to locate the sharp inflection point for tartaric acid. By comparison, the demonstration of the inflection point for the wine would require a more extended scale.

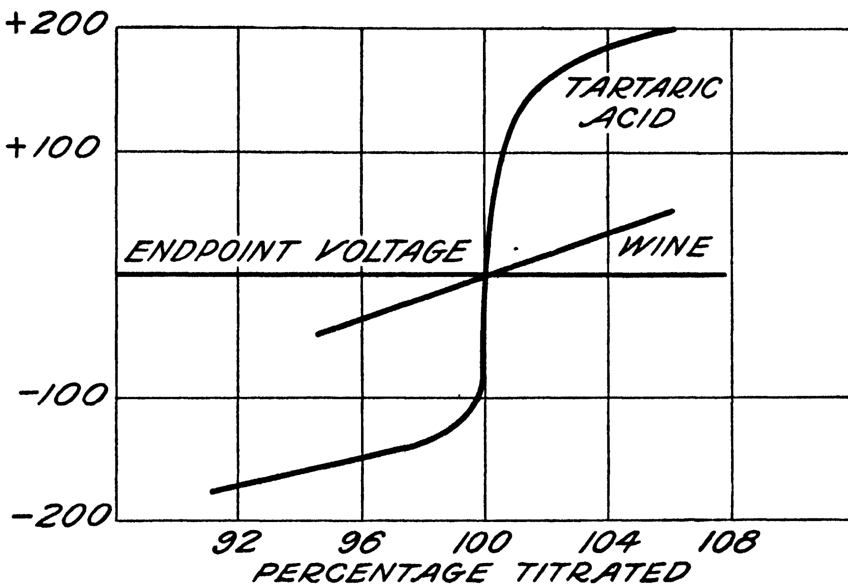

TABLE 1.—EXPERIMENTAL TITRATION CURVES (voltage readings in millivolts)

A mathematical treatment of the original data according to the method published by Fenwick[1] reveals the inflection point. This is summarized in Table 2. One critical postulate of this is that the volume additions over the end point be exactly alike.

By extending this treatment to data readily predictable, the effect of small errors in the volume additions may be studied. Table 3 shows the divergence in behavior between the wine and the tartaric acid. In this case the error is applied to the volume reading just before the inflection point and shows a range of volume error up to an accepted volume of one

[1] *Ind. Eng. Chem. Anal. Ed*, 4, 144 (1932).

drop of solution. In the case of the wine, the inflection point is shifted to another bracket by this sort of error, which explains the convergence of the upper curve. It is to be noted that errors of the same sort would apply to any system of observing changes progressing during titration, because of the buffered conditions near the end point.

While wine is a product difficult to titrate successfully, unpublished data of the Associate Referee indicate that all fruit products can be expected to show types of behavior lying between those of the examples shown in Table 1.

VOLUME	VOLTAGE	ΔE	$\Delta^2 E$	$\Delta^3 E$
15.40	140.2			
		−11.0		
15.60	151.3		+0.1	
		−11.1		
15.80	162.4		+0.9	+2.0
		−12.1		
16.00	174.5		−1.1	
		−11.0		
16.20	185.5			

$$D' = 0.9 \qquad D = 2.0 \qquad D^* = 1.6$$

$$\text{TITRATION VOL.} = 15.80 + \frac{0.9}{2.0} \times .20$$

$$= 15.89$$

TABLE 2.—ELECTROMETRIC TITRATION OF A WINE

P. S. Roller[1] has pointed out that the inflection point, while no doubt characteristic of a given acid solution, does not coincide with the stoichiometric end point for sufficiently weak acids. Further, the titration to a fixed *p*H can be made with greater precision and accuracy than an inflection point can be located. These conclusions may be applied to the data of Table 2. It may be noted that over the inflection point, 0.01 ml. of alkali solution corresponds to a change of 0.6 mv., or 0.01 *p*H, while the voltage reading may be made to 0.1 mv. even by the glass electrode here used. Therefore, if agreement can be reached as to the proper *p*H for the end point, titration to a fixed *p*H becomes a solution of the problem of acidity determination.

The Associate Referee recommends that titration of acidity in fruit products to a previously fixed end point be studied.

[1] *J. Am. Chem. Soc.*, **54**, 3485 (1932).

The Associate Referee will approve for publication any competent contributions that collaborators may make. He is very grateful for the collaboration of Donald H. Wheeler of the Bureau of Chemistry and

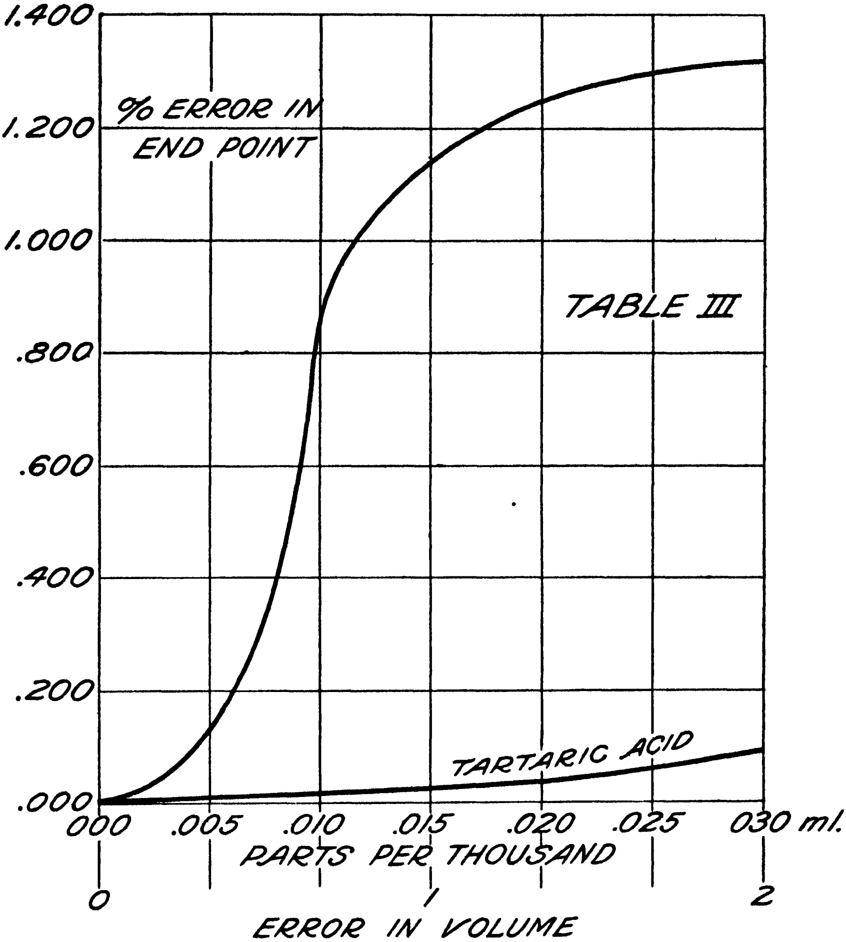

TABLE 3.—ERROR IN VOLUME (voltage readings in millivolts)

Soils, who kindly supplied from his studies on the electrochemistry of wines, the example used here to compare with the Associate Referee's data on tartaric acid.

No report on fruit acids was given by the associate referee.

REPORT ON VITAMINS

By E. M. NELSON (U. S. Food and Drug Administration, Washington, D.C.), *Associate Referee*

Two important contributions to the knowledge of the chemistry of vitamins have been made during the current year. Since there has been considerable speculation in the past as to the need for biological determinations of vitamins when their chemical nature becomes established, it seems desirable to give some consideration to these new developments. The chemical constitution of vitamin B_1 has been established, the synthesis of the vitamin has been accomplished both in this country and in Germany, and the synthetic vitamin is now available in commercial quantities. Vitamin D from a natural source has been isolated and shown to be identical in its characteristics to the product formed by irradiation of seven dehydrocholesterol.

Vitamin B_1 is a rather distinctive compound and may well lend itself to the formation of characteristic colored compounds that may serve as a measure of the quantity of the vitamin present. Availability of the pure vitamin will no doubt stimulate intensive research in that direction. The difficulties of determining vitamin B_1 by direct chemical methods can be appreciated when it is realized that good food sources of this vitamin, such as whole grains, contain approximately 1 mg. of the vitamin B_1 per pound.

A recent review on vitamin D states that there is evidence of the existence of eight forms of this vitamin. Theoretically the number may be even greater. These compounds are not of equal biological activity for one species, such as the rat, and some show distinctly different properties when fed to different species. There is a large number of sterols, all closely related chemically, some of which have the biological properties of vitamin D, and these compounds are so widely distributed that for the present the possibility of determining this vitamin in natural products by other than biological methods seems rather remote.

Two years ago the Association approved a recommendation for the appointment of a referee on vitamin C. In last year's report, however, attention was called to the fact that the International Standard for vitamin C had not become available until late in the year, and accordingly no referee was appointed. During the past two years a vast amount of literature has accumulated dealing with the chemical determination of vitamin C. One cannot review this literature critically without being impressed by the fact that the methods that have been used are not specific and that they have a rather narrow range of application. Until more specific methods are available they cannot be given serious consideration for adoption as official methods.

In recent years there has been very little activity in attempting to

improve biological methods for vitamin C determination. The available methods are cumbersome and usually yield results of a lower degree of accuracy than do assays of the other vitamins. Rather extensive researches may be necessary to establish the relative merit of different procedures in use, and unless this Association feels disposed to undertake this problem or the need for an official method become more urgent, it is believed to be desirable to give attention to other problems for the present.

Last year the report of the referee contained a recommendation for making available a standard cod liver oil for vitamin D assays. The purpose of this recommendation was to meet the need for a standard in assays with chicks. When this question was discussed informally with a group of feed control officials and representatives of the cod liver oil industry, the opinion prevailed that there was no danger of deterioration of the vitamin D of cod liver oil and that the cost of the U.S.P. reference cod liver oil as a standard made its use for chicks rather expensive. In view of the fact that the U.S.P. reference cod liver oil is also used as the international subsidiary standard for vitamin A, there was a further consideration that the supply of this oil might be exhausted too rapidly.

The Referee had previously sent to L. L. Lachat, Associate Referee on Technic and Details of Biological Methods, Vitamin D Carriers, a sample from a barrel of cod liver oil used in clinical studies conducted by the Children's Bureau, which were completed in the spring of 1935 and reported in the September number of the *Journal of Pediatrics*. This oil had been carefully assayed both against the International standard and the U.S.P. reference cod liver oil. It had shown no demonstrable change in potency over a period of 2½ years. Last December Lachat reported that the results obtained with the oil indicated a lower vitamin D content than had been assigned to it. Re-assay of the oil in the Referee's laboratory showed a marked reduction in vitamin D content. These observations led to the conclusion that any cod liver oil standard for vitamin D must be prepared and stored with great care to prevent deterioration and that frequent assays are necessary to assure a satisfactory standard. These conditions cannot be met without adding to its cost. Repeated assays have shown that the U.S.P. reference cod liver oil has maintained its vitamin potency without demonstrable loss, but if it is necessary to prepare and handle another standard with the same precaution the apparent advantage of having an additional standard would be greatly minimized.

Recent studies have shown that the total cost of vitamin D assays with chicks is increased by only a small percentage when the U.S.P. reference cod liver oil is used as a standard. The demand for this oil has not been so great as had been anticipated and there appears to be a sufficient supply to meet all demands for at least two years. For these reasons the Referee believes that a standard cod liver oil for chick assays, as

contemplated in last year's recommendation, should not be made available and he will act accordingly unless action of the Association directs otherwise.

One of the vitamins that has not been given serious consideration in the past appears to be of sufficient importance to warrant a study of methods for its determination. Reference is made to the substance that has been called vitamin B_2, vitamin G, and flavin. Sometimes the term "flavin" is prefixed by terms to indicate its source so that the name becomes "ovoflavin," "lactoflavin" and "hepatoflavin," for the flavins derived respectively from egg, milk, and liver. The naturally occurring flavins (d, ribityl-dimethyl isoalloxazine) appear to be identical in structure. A synthetic product alleged to be identical with the flavin that occurs in milk is now being manufactured by a pharmaceutical concern. Flavins have been shown to be a necessary constituent in the ration of rats and chicks. Recent investigations with poultry indicate that flavin may be a limiting factor in many practical poultry rations. Commercial whey is an important source of the vitamin and consideration has already been given to its manufacture from this material. If this practice does materialize, it will be important to have a convenient method for determining the flavin content of commercial preparations and also to determine whether products offered for sale as whey may have had the flavin extracted.

Chemical methods for the determination of flavin have been used and apparently with a fair degree of success. The concentration of the compound in a properly prepared extract is determined by measuring the intensity of fluorescence produced. It would be advantageous to have this subject discussed and to ascertain how important the determination of this substance may be, and also to find out if any members of the Association have the necessary equipment to enable them to carry out investigations on methods for the chemical determination of flavin.

The recommendations[1] made by the respective associate referees are approved.

REPORT ON VITAMIN A

DETERMINATIONS WITH THE HILGER VITAMETER

By J. B. Wilkie (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The absorption characteristic of vitamin A at 328 mμ has proved to be a rapid and dependable index of the quantity of this vitamin in a number of products. This measurement may be made with a conventional quartz or special laboratory spectrophotometer. This apparatus is costly,

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 54 (1937).

and the method is usually cumbersome and requires skilled manipulation. For this reason the Adam Hilger Company, British manufacturers of optical instruments, introduced a simplified apparatus for this purpose, known as the Vitameter. This instrument was described by F. W. Irish, the former associate referee, *This Journal*, **19**, 244 (1936). Further studies were made this year to determine its reliability.

Irish obtained results from eleven collaborators; the maximum variations from the average were between 21 and 47 per cent, depending upon the procedure used. The most satisfactory results were obtained when the Vitameter was standardized against U.S.P. cod liver oil, rather than when scale readings were accepted at their face value and a simple factor used.

One of the principal difficulties in operating the conventional Vitameter is the matching of dim flickering lines separated by a considerable distance. At least ten readings were necessary to obtain a good average. This fact, coupled with the non-availability of direct current necessary for the copper arc, led the Associate Referee to try a General Electric S-I lamp on the Vitameter. The lamp was equipped with a housing and a variable slit, with which arrangement a scale reading dilution curve could easily be plotted. The strips of light could be made brilliant, adjacent, and easy to read. With a little practice successive readings were duplicable. However, the scale readings could not be accepted as true E or extinction coefficient values as they formerly might have been because almost four times more oil was necessary to give the same reading. Undoubtedly the difference may be largely accounted for by a surplus of inadequately filtered light. Regardless of this disturbing condition, it was easily possible to follow vitamin A containing oil dilutions, and to plot reproducible curves of biological significance when adequate attention was given to the saponification and preparation of solution procedures.

The form of the dilution curve of an unknown oil will not always be similar to that obtained with the reference oil, but the biological tests do indicate a valuable approximate correlation. The changing slopes exhibited in the curve may indicate a failure of Beer's law or complications because of too much unfiltered light; however a curved line was obtained with the copper arc, though the curve was not so pronounced as when the S-I lamp was used. The use of this curvature, which has been almost universally observed, would obviously produce errors in calculating vitamin A concentration by multiplying the Vitameter extinction coefficient reading by 1600, as recommended by the Second International Vitamin Conference for computing true extinction coefficient values. Practically all the evidence collected by the Associate Referee from various Vitameters indicates the incorrectness of calculating the vitamin A content by multiplying the vitamin extinction coefficient values by 1600, or any other fixed factor. It is therefore believed that the most efficient use of

the Vitameter involves a dilution reference oil curve. The simple formula,

$$\frac{\text{Actual units/ml.} \times 100}{\%\ \text{dilution}} = \text{Vitamin A units per gram, is applicable.}$$

The readings on the Vitameter will vary with the position of the light source as much as one whole scale division, corresponding to a 0.1 density. It can thus be seen that with such a light source as a copper arc, which changes continually, a continually changing error will be obtained. Even with a relatively steady light, as the S-I is, extreme care must be taken to assure no appreciable error from this source. The uniformity of the filters is not beyond suspicion, and optical parts are subject to changes in transmission due to the light itself. The Vitameter, as well as any other probable simplified spectrophotometer for a similar purpose, is likely to have these weak points and changing characteristics. Therefore, if adopted such instruments, especially when involving extinction coefficient values, should be periodically checked by means of standards or recognized standard laboratory methods.

The difficulties with the vitameter mentioned may sound like a condemnation of this instrument, but this is not intended. Certain operators reported results that they stated would check recognized biological and spectrographic methods. The matter simply needs a more thorough investigation.

At present a solution to the problem seems to be the adoption of some laboratory method of spectrophotometry, possessed with certain requisite features and especially with provision for reliable self standardization. This may seem to be almost impossible for most prospective users, but there are indications that the existing interest will materialize into suitable and more economical apparatus.

Cod liver oil and other vitamin A containing material have irrelevant 328 mμ absorption, which can usually be eliminated by removing the saponifiable material. The following five steps to prepare the solution of the non-saponifiable fraction of an oil for the spectrophotometric examination are necessary: saponification, extraction, rinsing, evaporation, and final dilution procedure.

The experience of the Associate Referee has been that a more concentrated saponification solution and a longer period than that recommended by the British Pharmacopoeia and the original Vitameter instructions do no harm, but rather afford a factor of safety when working with unknown materials. The saponification procedure used at present is a modified Kerr-Sorber method, *This Journal*, **8**, 441 (1925), in which 5.0 grams of cod liver oil is saponified by being boiled with 30 ml. of ethyl alcohol and 3 ml. of 50 per cent potassium hydroxide for 20 minutes, whereas the original Vitameter procedure calls for a saponification of only 5 minutes with 10 ml. of 0.5 *N* alcoholic potash.

The second step of the extraction after saponification is carried out with such proportions of alcohol, ether, and water as to allow the original separation to be made with ease. A 1:1:1 ratio is satisfactory. One of the most perplexing difficulties encountered in this procedure was the tendency for emulsions to form during the third step, or rinsing, of the original ether solution. This difficulty was overcome by initially and alternately pouring water and N/6 potassium hydroxide solution through the ether layer without agitation until the persistent emulsification tendency was eliminated. Later rinses should have the customary agitation. R. S. McKinney of the Bureau of Chemistry and Soils stated that interfering quantities of soaps were difficult to remove during the rinses, therefore the latter operations were tested with hydrochloric acid, as the presence of turbidity, after the addition of this acid, indicates fatty acids and incomplete rinsing.

In the fourth step in preparing the final solution the washed ether layer is evaporated almost to dryness on the steam bath and finally to dryness on a constant temperature bath held at 50°C. Undoubtedly this evaporation is the most critical point in the whole procedure because vitamin A is most likely to be wholly or partially destroyed at this point by oxidation. An improvement over the present method during this evaporation might be the use of either an inactive or nonvolatile non-light absorbing solvent or an inert gas in place of absolute alcohol or cyclo-hexane, which have been used most generally. Absolute alcohol is a poor solvent, especially for some of the halibut liver oils. Cyclo-hexane has a strong tendency to form emulsions with a commonly occurring trace of unavoidable moisture. This emulsion is strongly absorbing in the ultraviolet and thus seriously interferes with the determination. For most purposes a mixture of 90 per cent alcohol and 10 per cent of cyclo-hexane avoids both of these difficulties. An 80 per cent alcohol—20 per cent cyclo-hexane mixture is even a better solvent, but it is more expensive and is generally not necessary.

As it is well known that vitamin A is susceptible to deterioration from light, the operations described are usually carried out in a subdued light. However, gross deterioration from the ordinary light encountered in the laboratory has not been noted. The real effect of light during the preparation of the vitamin A solution remains to be determined.

PROCEDURE

The specific procedure based upon the foregoing considerations is as follows:

Boil 1 gram of the cod liver oil with 30 ml. of 95% alcohol and 3 ml. of 50% KOH for 20 minutes. Maintain the initial volume with a reflux condenser or by adding alcohol during the boiling. Cool, and add 20 ml. of water. Transfer the mixture to a separatory funnel and extract with three 30 ml. quantities of diethyl ether.

Combine these ether extracts in a separatory funnel and pour 100 ml. of water without agitation through the ether layer. Discard the water and wash without agitation with 100 ml. of water. Pour 15 ml. of $N/6$ KOH through the ether layer, discarding the clear aqueous solution. Add 100 ml. of water without agitation and again discard the clear aqueous layer. Alternately continue the water and $N/6$ KOH rinses until any emulsion formed is of a very transient nature. Make subsequent washings with agitation. Rinse three times with $N/6$ KOH, neutralize the last of these rinses with 10% HCl, and examine for turbidity. If necessary continue the $N/6$ KOH rinses until there is no appreciable turbidity when the 10% HCl is added. Rinse three times with water. Allow the final rinse to stand 5 minutes. Separate the water drainage.

Evaporate the final ether extract almost to dryness on the steam bath. Finish the evaporation on a 50°C. constant temperature water bath, taking care to leave the dried residue exposed no longer than 2 minutes on the water bath. Make to volume with absolute ethyl alcohol or 10% cyclo-hexane—90% alcohol solution for spectrophotometric examination.

During the past year the Associate Referee has had occasion to determine the 328 mμ absorption of the unsaponifiable portion of condensed milk. The extraction of the contained fat was found to be very difficult. A modification of the Rose-Gottlieb method was finally found to be workable. The quantities of reagents and the periods necessary for good separation were found to be critical. Owing to its possible value, this method will be presented in this report. It follows:

FAT EXTRACTION AND VITAMIN ESTIMATION FOR EVAPORATED MILK

Measure into a 300 ml. separatory funnel 100 ml. of evaporated milk. Add 120 ml. of 95% alcohol and shake 1 minute. Allow to stand 5 minutes. Add 20 ml. of NH_4OH and shake 1 minute. Allow to stand 5 minutes. Add 100 ml. of petroleum ether and shake 1 minute. Allow to stand 1 minute. Separate the solutions. Add 50 ml. of petroleum ether to the aqueous portion and shake 1 minute. Allow to stand 5 minutes and separate. Again add 50 ml. of petroleum ether to the aqueous portion and shake 1 minute. Allow to stand 5 minutes and separate. Combine all the petroleum ether extracts, discarding the aqueous portions that may settle out. Run the petroleum ether extract into a 200 liter beaker and evaporate to the cessation of ebullition. Saponify by boiling for 20 minutes with 50 ml. of alcohol and 15 ml. of 50% KOH. Make to 40 ml. with alcohol, then to 80 ml. with water. Extract with three 25 ml. portions of ethyl ether. First rinse in a separatory funnel with 5 ml. of 0.5 N KOH and 50 ml. of water. Rinse alternately with 5 ml. of 0.5 N and 15 ml. of $N/6$ KOH. Use a few drops of alcohol if small layers of emulsions form at the interfaces during the caustic washing. Test the caustic rinsings until no turbidity occurs when the rinsing is acidulated with 10% HCl. Rinse with three 50 ml. portions of distilled water. Evaporate the ether extract, first on the steam bath to the cessation of ebullition and finally on the 50°C. constant temperature bath for 5–15 minutes, depending upon the appearance. Take up with a minimum quantity of a 90% alcohol—10% cyclo-hexane mixture and make to 10 ml. for the spectrophotometric examination.

It is recommended[1] that spectroscopic methods for the determination of vitamin A be further studied.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 54 (1937).

REPORT ON VITAMIN D

DEVELOPMENT OF A METHOD FOR THE ASSAY OF VITAMIN D IN MILK—DURATION OF THE ASSAY PERIOD*

By WALTER C. RUSSELL (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

Consideration of the preliminary results reported for 1935, *This Journal*, **19**, 248 (1936), led to the proposal that a study be made of the duration of the assay period before further work is done on the projects already outlined. The proposal was to determine the number of days required to reach a maximum response in assay animals and to use this information to arrive at an assay period of less than 10 days' duration, if possible. If a shorter period of assay seemed feasible, it was to be tried by a group of collaborators in comparison with the 10-day procedure.

PROJECTS

6.—Duration of the assay period

In order to determine the number of days required to reach a maximum healing response the following experimental procedure was used. The calculated quantity of milk used for the trial was fed in 3 equal portions to a group of 50 rachitic rats for the first 3 days of the assay period. The total quantity of milk fed was that which would be administered during the first 8 days of the usual 10-day assay period. Groups of 10 rats each were killed, and the line test was observed in the radii at the end of the 4th, 6th, 8th, 10th, and 12th days.

Six laboratories participated in the project, and the results are shown in Table 1. In the case of the concentrate milks maximum responses were noted at 10 days, and the value at 12 days was slightly lower in each case

TABLE 1.—*Average numerical responses**

DURATION OF ASSAY PERIOD	LABORATORY A CONCENTRATE MILK. FED AT 8 CC. LEVEL	LABORATORY B CONCENTRATE MILK. FED AT 7.5 CC. LEVEL	LABORATORY C CONCENTRATE MILK. FED AT 8 CC. LEVEL	LABORATORY D CONCENTRATE MILK. FED AT 6.75 CC. LEVEL	LABORATORY E IRRADIATED MILK. FED AT 18.9 CC. LEVEL	LABORATORY F IRRADIATED MILK. FED AT 30 CC. LEVEL
days						
4	0.36	0.10	0.0	0.0	0.89	0.18
6	0.34	0.37	0.05	0.39	1.05	0.65
8	0.43	0.50	0.20	0.75	1.8	0.65
10	0.61	0.80	0.55	0.50	1.7	0.63
					1.7†	
12	0.50	0.70	0.45	1.0	2.0	0.88

* Radii were sent to the laboratory of the associate referee, where they were scored, and an average response was calculated, the following numerical values being used:
−, 0; −(±), 0.25; ±, 0.50; ±(+), 0.75; +, 1; +(++), 1.5; ++, 2; +++, 3; ++++, 4.
† Incorporated in food.

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

with the exception of Sample D. The responses were read with difficulty in the case of this sample, however, because either poor rickets prevailed, or the degree of cure was too great to permit satisfactory reading of the response. On the other hand, the responses shown at 12 days by the 2 irradiated milks were slightly higher than the response at 10 days. With the exception of Sample C, responses of a definite degree were noticed as early as the 6th day.

While the data are not extensive enough to permit a conclusion to be drawn as to the length the assay period should be, if it is to be made shorter than 10 days they indicate that 6, 7, or 8 days might be used. Of these possibilities a 7-day period is the most convenient. The response at 7 days would be expected to be weaker than that at 10, and if the sample under examination were of low potency, or if the rats showed an increased resistance to cure, a sufficiently strong response might not be obtained at 7 days. This would seem to be the principal objection to the use of a 7-day period, but it could be met to a large extent by the feeding of a higher level of milk and of reference oil.

7.—Comparison of a 7-day with a 10-day assay period

The results of Project 6 indicated that a 7-day period might prove feasible, and therefore it seemed desirable to determine whether an assay period of this length would give results that would permit the same interpretation to be made as when a 10-day period is used. It is not necessary, however, that the same response be obtained at the end of 7 days as at 10, so long as the calcification is marked enough for scoring and is of the order of a narrow continuous line. The following procedure was proposed. A sample of milk with corresponding reference oil was to be fed the usual 8 separate daily feedings and the animals killed at the end of a 10-day assay period. To another group, having essentially the same litter representation, an equal quantity of the same sample of milk was to be fed as 3 separate daily feedings for the first 3 days of the period, and the animals were to be killed at the end of 7 days. Along with the milk the reference oil was to be fed in a parallel manner.

Comparisons were made by collaborators who are conducting routine assays of milk according to the 10-day assay period. Nine laboratories collaborated and reported results on 12 samples of milk. On account of rickets-resistance or the feeding of too high levels, so that almost complete cures resulted instead of lines of calcification, it was not possible to interpret the results from 5 of the laboratories. This observation emphasizes the difficulties involved in this type of work and the presence of factors, such as rickets-resistance, which are frequently beyond the control of those in charge of laboratories. In Table 2 the responses obtained by the laboratories whose results are interpretable are shown. The following observations are noted.

TABLE 2.—*Comparison of 7-day with 10-day responses**

LABORATORY	PERIOD	REFERENCE COD LIVER OIL	VITAMIN D MILK	INTERPRETATION OF RESULTS
A	7-day	0.30	0.8	Milk not less than specified potency
	10-day	0.45	1.1	Milk not less than specified potency
B	7-day	0.65	1.5	Milk not less than specified potency
	10-day	1.25	1.3	Milk not less than specified potency
C	7-day	0.72	0.63	Milk essentially same as specified potency
	10-day	0.88	0.85	Milk essentially same as specified potency
D(1)	7-day	0.78	0.65	Milk essentially same as specified potency
	10-day	0.63	0.90	Milk not less than specified potency
(2)	7-day	0.78†	0.90	Milk not less than specified potency
	10-day	0.63	1.3	Milk not less than specified potency
(3)	7-day	0.15	0.18	Response too weak for interpretation
	10-day	0.93	0.13	Milk less than specified potency
(4)	7-day	0.15	0.58	Milk not less than specified potency
	10-day	0.93	0.98	Milk not less than specified potency

* The numerical values in the table are average responses.
Radii were sent to the laboratory of the associate referee, where they were scored, and an average response was calculated, the following numerical values being used:
−, 0; −(±), 0.25; ±, 0.50; ±(+), 0.75; +, 1; +(++), 1.5; ++, 2; +++, 3; ++++, 4.
† The same reference oil group was used for D-1 and D-2.

In 5 of the 7 cases, the interpretation at 7 days is the same as that made at 10 days. In one instance, D-1, for the 7-day period the interpretation was "essentially the same as specified potency," whereas for the same milk at 10 days the interpretation was "not less than." The response at 7 days in the remaining instance, D-3, was too weak to permit a satisfactory reading. At 10 days, however, the milk was definitely less than the required potency.

With only one exception, D-1, the response was less when the reference oil was fed for 3 days and the animals killed at 7 days, than when 8 feedings were made and the animals killed at 10 days. For five of the milk samples the 7-day response was less than the 10-day. Two samples, B and D-3, showed essentially the same response for both assay periods. On account of the weaker response at the end of a 7-day as compared with a 10-day period, it would be advisable to feed higher levels of supplements if a 7-day period is used. In some laboratories it may not be possible to administer the total volume of irradiated milk in a period of 3 days and therefore it is suggested that the feeding period be extended to 5 days.

Although the procedure for the assay of vitamin D milk has not been

studied as much as is desirable, it seems advisable at this time to propose a tentative method for the assay of vitamin D milk, with certain options as to the method of administering supplements and the duration of the assay period.

As soon as a procedure is agreed upon, a test of accuracy and reproducibility should be made. This could be accomplished by each of several laboratories conducting 10 assays per laboratory at approximately the same time, on the same sample of specially prepared, evaporated milk.

The method recommended for adoption as tentative[1] was published in *This Journal*, 20, 78 (1937).

The Associate Referee appreciates the splendid cooperation of the collaborators.

REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

As Associate Referee on Tomato Products for the past year, F. Leslie Hart submitted a report on the determination of chlorides in tomato juice. No other work was done on canned foods during the past year.

In the new edition of *Methods of Analysis* it is noted that the method for the determination of moisture in canned vegetables refers to a method in a previous chapter that provides for drying over sulfuric acid in a desiccator. Examination of previous reports shows that there was no determination of solids given for canned vegetables in Bulletin 107. In the first edition of *Methods of Analysis* drying for 10 hours at 100° is given as the official method. In the second edition drying in vacuum oven at 70° to constant weight is given as official. The third and fourth editions refer to the paragraph in the chapter on grains and stock feeds, which calls for drying without heat over sulfuric acid. A review of the proceedings shows there has been no study of moisture in canned vegetable products, with the exception of studies on moisture in tomato purée made last year. There seems to be no foundation for recognition of the method of drying over sulfuric acid for canned vegetables.

RECOMMENDATIONS[2]

It is recommended—

(1) That the method for the determination of chlorides in tomato juice presented by Associate Referee Hart, *This Journal*, 20, 78 (1937), be adopted as tentative.

(2) That studies of methods for determining quality factors and fill of container be continued.

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 54 (1937).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 58 (1937).

(3) That studies of methods for the analysis of tomato juice be continued.

(4) That paragraph 3, chapter XXXV, *Methods of Analysis*, 1935, be changed to read "Total solids—tentative. Proceed as directed under 16."

REPORT ON TOMATO JUICE

I. DETERMINATION OF CHLORIDES

By F. Leslie Hart (U. S. Food and Drug Administration, Buffalo, N. Y.), *Associate Referee*

The only method for the determination of chlorides in tomato juice that has been approved by the Association is that given in *Methods of Analysis*, 1935, p. 500, XXXV, 22. This method involves ashing in the presence of sodium carbonate, with subsequent extraction of soluble chlorides with hot water and nitric acid. The chlorides are hen determined either gravimetrically or by the Volhard volumetric procedure. The method, originally adopted for the determination of chlorine in plants, has been approved, either as an official or as a tentative method, for various products, for example, eggs and egg products, fruits and fruit products, vegetables and vegetable products, wines, coffee, meat extracts, alimentary pastes, and bread.

Procedures for the determination of chlorides that involve ashing as a preliminary step have been subject to criticism. Tilden, as Associate Referee on Total Chlorine in Plants, devoted much study to this problem. In her report for 1928, *This Journal*, 11, 209 (1928), she concluded that ashing plant material in the presence of a chlorine fixative appears to be the only practical method, and recommended the use of sodium carbonate. A later report, *Ibid.*, 12, 195 (1929), involving collaborative work on pineapple juice, showed discrepancies in the reported amounts of chlorine, which were attributed either to variations in the sample itself, or in the preparation of the ash. In the reports cited, Tilden devoted some attention to methods involving "wet ashing," and recommended further investigation. Further objections have been raised against procedures for the determination of chlorine involving preliminary ashing, because of the length of time required.

Various modifications of wet digestion methods for chlorine may be found in the literature, chiefly on physiological products. Lawrence and Harris[1] devised the method now known as the Open Carius Method. This method was chosen for this study as a preliminary treatment of the sample before titration of the chlorides. After any preliminary treatment required for the destruction of organic matter, the analyst has a choice

[1] *J. Am. Chem. Soc.*, 46, 1471 (1924).

of methods for the final determination of chlorides. The three methods attracting the most attention are: gravimetric determination as silver chloride, direct titration with standard silver nitrate as described by Gay-Lussac, and the Volhard method. The first two methods, while accurate, are tedious. The Volhard method has received official recognition, as mentioned previously.

The Volhard titration has been subject to critical analysis by Kolthoff and Menzel[1] and Kolthoff and Sandell.[2] These authors call attention to the fact that thiocyanate acts on the precipitated silver chloride, resulting

Results of Collaborators
(Present, 0.904 g. NaCl/100 cc.)

ANALYST	FILTERED JUICE	UNFILTERED JUICE
	grams NaCl per 100 cc.	
D. W. McLaren*	0.91 (av. of 6)	0.89 (av. of 3)
M. G. Yakowitz	0.90 (av. of 3)	0.88 (av. of 3)
G. A. Pitman	0.91	0.79†
L. H. Feldstein	0.89	0.89
	0.90	0.89
H. Shuman	0.91	0.89
	0.91	0.90
H. I. Macomber	0.90	0.88
	0.90	0.88
A. E. Mix	0.91 (av. of 6)	0.89 (av. of 2)
F. Leslie Hart	0.90 (av. of 4)	0.90 (av. of 2)

* McLaren also determined chlorides on the filtered juice by the usual Volhard procedure, in which the precipitated chlorides were filtered off before titration. He reports 0.91 (0.90) g/100 cc. by this procedure.
† See Comments of Collaborators.

in a fading end point. The silver chloride readily absorbs silver ions. This error is stated by Kolthoff to be about 1 per cent when the excess of silver nitrate is removed either by filtering or pipetting from the precipitated silver chloride. He also mentions the fact that filter paper has an appreciable absorptive action.

The side reaction of silver chloride and excess thiocyanate is relatively slow. Various procedures have been recommended to further retard this reaction. That divised by Caldwell[3] was used in the method sent to col-

[1] Volumetric Analysis, Vol 1, p. 167; Vol. 2, p. 227 (1928).
[2] Textbook of Quantitative Inorganic Analysis, p. 454 (1936).
[3] *Ind. Eng. Chem. Anal Ed.*, 7, 38 (1935).

laborators by the Associate Referee. It directs the addition of 1 cc. of nitrobenzene immediately before back titration with thiocyanate solution.

A quantity of unsalted tomato juice processed from a batch of 5833 cc. was obtained at a cannery. This was taken directly from the extractor, 50 grams of purified salt was added to the juice, and the resulting mixture was transferred to No. 1 cans, capped, and processed according to the usual cannery procedure. These cans were sent to the collaborators with a copy of the method recommended. The method has been published, *This Journal*, **20**, 78 (1937).

COMMENTS OF COLLABORATORS

Shuman.—No difficulty with the method. Filtering is unnecessary.

McLaren.—No difficulties encountered. Nitrobenzene gives a sharp end point.

Pitman.—Believe value reported for unfiltered juice inaccurate because of drifting end point during last 2 cc. of titration.

Yakowitz.—Difficult to obtain a sharp end point on the unfiltered juice. Additional nitrobenzene caused no improvement.

DISCUSSION

The salt content of the original juice was determined by two methods:

Official Method—0.047 (0.044) g. NaCl/100 cc.
Proposed Method—(Av. of 6 determinations) 0.047 g. NaCl/100 cc.

There was therefore present in the samples sent to collaborators 0.904 g. NaCl/100 cc. It will be noted that the results obtained by all collaborators except one agree quite well with this figure. It is believed that the low result is due to some unnoticed deviation from the outlined procedure, possibly either insufficient nitric acid or insufficient boiling. The results reported for the filtered juice average slightly higher than those for the original unfiltered juice, but the difference is too small to be of any significance.

The determination of salt in tomato juice is made most frequently in connection with the estimation of soluble solids content by the refractometer, in which a correction for salt content is necessary. For this reason directions to collaborators called for analysis of a measured volume of the filtered juice. The method proposed to the Association provides for either a weighed or measured amount of the filtered or unfiltered juice.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method discussed be adopted as a tentative method.

(2) That studies of methods for the analysis of tomato juice be continued.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 58 (1937).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

During the past year the work of the Referee has been chiefly of an advisory nature. He has, however, made a digest of the publications that have dealt with the so-called "quick tests" advanced for the determination of ample, intermediate, and inadequate supplies of "available" plant nutrients, *i.e.*, N, P, and K, with possible extension to include other elements. The Referee is not convinced, however, that the time is propitious for a recommendation relative to a more active participation in this line of work.

It is recommended[1]—

(1) That collaborative study be made of the ammonium chloride distillation method for the determination of exchangeable bases and saturation capacity of soils.

(2) That the determination of *p*H values in the soils of the arid and semi-arid regions be studied further and that particular attention be directed toward the adaptability of the glass electrode.

(3) That the Associate Referee on *p*H Value in Soils of the Humid Regions likewise study the adaptability of the glass electrode.

(4) That work be continued on the determination of selenium in soils.

(5) That work on liming materials be continued.

(6) That the fusion and the volatilization methods for the determination of iodine in soils, *Methods of Analysis, A.O.A.C.*, 1935, 8–10, be adopted as official (first action).

(7) That the Associate Referee on Less Common Elements be requested to pursue a study of methods for the determination of fluorine in soils.

REPORT ON HYDROGEN-ION CONCENTRATION OF ALKALINE SOILS

By W. T. McGeorge (Agricultural Experiment Station, Tucson, Ariz.), *Associate Referee*

Following up the report of P. L. Hibbard, submitted last year, *This Journal*, **19**, 256 (1936), the present Associate Referee is largely in agreement with his recommendations and suggestions.

The *p*H of alkaline soils is being studied as a major project at the Arizona Experiment Station. This investigation has shown that the glass

[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 52 (1937).

electrode is most desirable for alkaline soils, both for accuracy and speed. On the other hand, accurate results can be consistently obtained by means of the hydrogen electrode if precautions are taken to eliminate carbon dioxide from the system.

An important outcome of the investigation is one that shows that the high *p*H of alkaline soils is in large part due to calcium carbonate. The *p*H of pure calcium carbonate is 9.3–9.6.

The great error arising from contact with carbon dioxide during the *p*H determination on soils is caused by absorption of carbon dioxide and conversion of calcium carbonate to bicarbonate. Since calcium carbonate is soluble only to the extent of 6–8 p.p.m. it is readily seen that very little carbon dioxide would be required to reduce materially the *p*H of calcium carbonate solution.

It is the opinion of the Associate Referee that cooperative work should be conducted upon a study of the sources of error that commonly arise in the *p*H determination on alkaline soils. One of these is the error introduced by the time factor, that is, how soon after mixing the soil and water should the *p*H reading be taken? Base exchange reactions will cause an increase or decrease in *p*H with time, depending upon the nature of the soluble salts present in the soil. In alkaline calcareous soils it is usually a reduction in *p*H. Another important source of error is the soil-water ratio. Lowest *p*H values are obtained by the lowest workable soil-water ratio and this value approaches, most closely, the *p*H of the soil in the field. As the soil-water ratio is increased, the *p*H rises until the dilution reaches the point of maximum hydrolysis. This represents the maximum potential alkalinity of the soil and is usually a constant figure. Agreement on methods depends upon whether the objective is the *p*H of the soil in the field or the maximum alkalinity of the soils. It is of interest that results obtained in this laboratory show that *p*H values obtained by using tap water instead of distilled water for the soil suspension agree closely with the *p*H of the soil solution as obtained by displacement methods.

Cooperative experiments along the above lines are being planned for the coming year, and it is recommended[1] that the work be continued.

No report on the hydrogen-ion concentration of acid soils was given by the associate referee.

No report on liming materials was given by the associate referee.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 52 (1937).

REPORT ON LESS COMMON ELEMENTS IN SOIL

By J. S. McHARGUE, *Associate Referee* and W. T. FORSEE
(Kentucky Agricultural Experiment Station, Lexington, Ky.)

During this year further collaborative work was done on the fusion and combustion methods for the determination of iodine in soil.

Two samples of soil, A and B, were furnished by J. H. Mitchell of the South Carolina Experiment Station for the determination of iodine. The results obtained by the collaborators on these and also on other soils are presented in Table 1.

COMMENTS ON RESULTS REPORTED

From the figures shown in Table 1 it will be observed that on the samples of South Carolina soils A and B fairly concordant results were obtained by four of the analysts. The results obtained by Ford on South Carolina soil B ranged from 6.2 to 9.04 p.p.m. of iodine. The result 9.04 p.p.m. is regarded as low in comparison with those obtained by the other analysts.

The results reported on the Fayette County soil do not agree very well. Shaw and Forsee differ by 0.8 p.p.m. Ford's result of 3.1 p.p.m. is low. On the sample of Tennessee soil A, Forsee obtained 1 p.p.m. less iodine than Shaw. On Tennessee Soil B Forsee obtained only 0.7 p.p.m. less iodine than Shaw, which is very good agreement for two analysts on the same sample.

On the Kentucky sandy loam soil Ford made two determinations, using 5 gram samples in contrast with two determinations using 10 gram samples and obtained closely agreeing results in each case, but the average for the 10 gram samples was 0.4 p.p.m. less iodine than was obtained for the average of the two 5 gram samples.

On the Kentucky clay loam soil Forsee obtained 1.5 p.p.m. less iodine than was obtained by Shaw, which is in accord with their other results, namely that Forsee's were consistently lower by about 1 p.p.m. The results obtained by Forsee on some of the samples of soil with the combustion method agree rather closely with those obtained by the fusion method.

CHANGES IN METHODS

The fusion method as described at the 1935 meeting and later published in *This Journal*, **19**, 66 (1936), has been revised to eliminate the use of sodium sulfite as a reducing agent because it was found that this reagent is not necessary. It is also advisable to heat the iron crucible containing the fused mass of soil in the electric furnace at a temperature of 400°C. for about 10 minutes to burn any organic matter that may be unconsumed in the fusion of the soil.

For the combustion method it has been found to be desirable to use

TABLE 1.—*Collaborative results*

ANALYST	SAMPLE	IODINE	
	Fusion Method		
	S. C. Soil A		p.p.m
McHargue	a		1.6
	b		1.6
	Av.		1.6
Mitchell			1.66
	S. C. Soil B		
McHargue	a.		11.2
	b.		14.3
	Av.		12.75
Mitchell			12.35
Shaw*			11.5
Forsee			10.8
Ford†			9.04
	Fayette Co., Ky. soil (average amount of I)		
Shaw			5.0
Forsee			4.2
Ford			3.1
	Fayette Co., Ky. clay loam (relatively large amount of I)		
Shaw			13.9
Forsee			12.4
	Tenn. soil A (low amount of I)		
Shaw			2.1
Forsee			1.1
	Tenn. soil B (average amount of I)		
Shaw			7.5
Forsee			6.8
	Laurel Co., Ky. sandy loam (low in I)		
Forsee			0.93
Ford		*5 grams*	*10 grams*
		1.5	1.2
		1.6	1.2
	Av.	1.6	1.2
	Combustion Method		
Forsee, Fayette Co. soil			4.2
Forsee, S. C. soil B			10.5
Forsee, Tenn. soil A			1 1
Forsee, Tenn. soil B			6.7

* Tenn. Agr. Expt. Station.
† Ind. Agr. Expt. Station.

a 0.001 solution of sodium hydroxide to absorb the vapors of iodine in the Milligan absorption bottles instead of a 5 per cent solution of potassium carbonate.

Because turbidity was experienced at times in the carbon disulfide iodine extract in both the fusion and combustion methods the writers

made some experiments to ascertain whether or not iodates are present, as has been assumed by previous investigators. Determinations of iodine in portions of the same sample of soil were made by both methods. In five determinations by each method it was assumed that iodates were present, and sodium acid sulfite was used to reduce them to iodides. In the remaining five determinations on the same soil by each of the methods, it was assumed that iodates were not present, and accordingly no sulfite was used. The results are shown in Table 2.

TABLE 2.—*Results that indicate the absence of iodates in the fusion and combustion procedures for the determination of iodine in soil*

SAMPLE	VOLATILIZATION METHOD	ABSORBED IN DILUTE NaOH	FUSION METHOD	
	REDUCED WITH SULFITE	NO SULFITE ADDED	REDUCED WITH SULFITE	NO SULFITE ADDED
1	3.96	4.16	4.22	4.16
2	4.03	4.04	4.37	4.52
3	4.31	4.24	4.05	4.30
4	4.34	4.44	4.33	4.12
5	4.16	4.12	4.17	4.08
Av.	4.16	4.20	4.23	4.24

The results in Table 2 indicate that iodates are not formed in the fusion and combustion procedures for the determination of iodine in soil. Therefore the sulfite is not necessary. More accurate determinations may be obtained by omitting this reagent, because it apparently is the cause of turbidity in the carbon disulfide iodine extract when added as a reducing agent.

To ascertain the relative merits of carbon disulfide and carbon tetrachloride as mediums in which to develop the iodine color, a comparison was made of their color intensities with varying amounts of iodine. The same volume of a standard potassium iodide solution was transferred to two 30 ml. separatory funnels containing 20 ml. of water. The iodine was liberated with measured quantities of reagents, extracted from one solution with 1 ml. of carbon disulfide and from the other with 1 ml. of carbon tetrachloride, and the colors were compared in a microcolorimeter. The results (Table 3) are the average of three comparisons for each iodine concentration and show a relative color intensity of about 7 to 5 for iodine extracted with carbon disulfide and carbon tetrachloride. For quantities of iodine less than 0.00005 gram the writers have found carbon disulfide to be much superior to carbon tetrachloride. However, for 0.00005 gram or more, carbon tetrachloride serves equally as well, but it is only preferable in very warm weather.

TABLE 3.—*Comparison of the intensity of colors in carbon disulfide and carbon tetrachloride*

IODINE	COLOR INTENSITY CS_2/CCl_4	REMARKS
gram		
0.000005	1.50	Color in CCl_4 was not perceptible until placed in the colorimeter. Color in CS_2 was perceptible previous to placing in the colorimeter.
0.00001	1.42	Color in CCl_4 was barely perceptible before placed in the colorimeter. Color in CS_2 was satisfactory for reading.
0.00002	1.34	Color in CCl_4 not satisfactory for reading. Color in CS_2 was satisfactory for reading.
0.00004	1.36	Color in CCl_4 not entirely satisfactory for reading. Color in CS_2 satisfactory for reading.
0.00005	1.38	Approximately the limit at which accurate determinations can be made in CCl_4.

Care should be taken in regard to the amounts of reagents used in developing the iodine color, and the two separatory funnels should contain approximately the same volumes of water. The solutions in the separatory funnels should be allowed to stand about 2 minutes after acidifying in order to allow the carbon dioxide to escape. The same volume of sodium nitrite solution should be added to both the standard and the unknown. The writers have found a large excess of sodium nitrite solution to be very detrimental because it colors the carbon disulfide layer and alters the shade of color produced by iodine. This color change is probably due either to dissolved nitric oxide or to bromine that might be liberated by excess sodium nitrite. Five drops of a 10 per cent sodium nitrite solution is ample for a determination.

The Associate Referee wishes to recommend[1] that the fusion and combustion methods for the determination of iodine in soil be adopted as official (first action).

REPORT ON SELENIUM IN SOILS

By KENNETH T. WILLIAMS (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

Gooch and Pierce[2] based a process for the separation of selenium and tellurium on the volatility of selenium tetrabromide and the non-volatility

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 52 (1937).
[2] *Am. J. Sci.*, 4, 181 (1896).

of tellurium tetrabromide from a solution of phosphoric acid containing potassium bromide. Later, distillation with hydrobromic acid and bromine was used to separate selenium, arsenic, and germanium from the other elements.[1] Robinson *et al.*[2] applied this method of separation to soils, shales, ores, and organic matter after their proper preparation. Since Robinson's publication appeared the Division of Soil Chemistry and Physics has determined selenium in approximately 5000 soil samples. Experience has made possible certain changes and additions to improve the accuracy of the method. A description of the method now used is given here, together with some analytical data.

MODIFIED METHOD FOR DETERMINATION OF SELENIUM

DISTILLATION

Place 50 grams of air-dry soil that has passed a 2-mm. sieve in the distilling flask (see *Methods of Analysis, A.O.A.C.*, 1935, 14). Add to the contents of the flask, slowly and with constant shaking, 100 ml. of 48% HBr containing 2–3 ml. of Br_2.

After any frothing due to decomposition of carbonates has subsided, connect the still so that the adapter is below the surface of the 5 ml. of Br_2 water in the receiving flask. (The success of the distillation depends on the saturation of the organic matter in the soil with Br_2 so that it will not reduce the $SeBr_4$ during the distillation.) Apply heat gradually and when the mixture begins to boil adjust the flame so that the distillation proceeds very slowly (approximately 20 minutes for 1–2 ml. of bromine), thereby giving time for the Br_2 and soil organic matter to react. If sufficient Br_2 was not added to the soil to give 1–2 ml. in the distillate, remove the flame and add more Br_2 in HBr solution through the thistle tube. (After this period of bromination, the distillation may be finished rapidly.) Collect 50–60 ml. of distillate. To check the complete distillation of the selenium, add more HBr and Br_2 to the residue and collect a second distillate. Remove the receiving flask, dilute the distillate to 80–90 ml. with distilled water, and pass in SO_2 until the Br_2 is reduced and the solution becomes essentially saturated with the SO_2. (If the reduction is made in concentrated acid and the amount of selenium is small, it often precipitates as a yellow, finely-divided form that is exceedingly hard to retain on a filter mat.) Keep the solution cool during the reduction with SO_2 to avoid possible loss by volatilization just as the reduction of the Br_2 is completed.[3] Add 0.3–0.5 gram of $NH_2OH \cdot HCl$ and warm on the steam bath for 20 minutes.

ESTIMATION

Set the distillate aside for 48 hours to allow complete reduction and coagulation of the selenium. Examine the distillate for selenium; if the amount is small, employ the Tyndall effect, using either direct sunlight or a strong, well-focused, artificial light. As little as 0.005 mg. may be easily seen by this method. Filter the selenium on a tight asbestos mat in a Gooch crucible, wash with cold water then with alcohol to remove the oily material always present in soil distillates, and again wash with cold water. If the amount of selenium is 0.5 mg. or less, dissolve on the pad with 10 ml. of water-white* 48% HBr to which has been added 3 ml. of Br_2 per liter. Filter into a 25 ml. volumetric flask and wash the pad with cold water until the volume reaches 24 ml. Pass in SO_2 until the solution is essentially saturated. Add

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[1] Noyes and Bray, Qualitative Analysis for the Rare Elements. The Macmillan Co., New York (1927).
[2] *Ind. Eng. Chem. Anal. Ed.*, 6, 274 (1934).
[3] Hillebrand and Lundell, Applied Inorganic Analysis. John Wiley and Sons, New York (1929).
* Water white or that will become water white when diluted with an equal volume of water and treated with SO_2.

1 ml. of a fresh solution of $NH_2OH \cdot HCl$ containing 10 grams per 100 ml. of solution. Shake well. Heat on the steam bath for 20 minutes, bringing the temperature to 45–50°. Cool to room temperature, shake well, and compare in Nessler tubes with known amounts of selenium precipitated in the same manner. Prepare fresh standards each time. (In routine work it is convenient to accumulate 25–50 samples for estimation.) Then prepare standards containing 0.01, 0.025, 0.05, 0.10, 0.15, 0.20, 0.30, and 0.50 mg. of selenium from a standard stock solution of Na_2SeO_3 or Na_2SeO_4. (By treating each sample and the standards exactly alike in the manner described, the colored turbidities can be readily matched. In spite of all precautions, an off-colored one will be obtained from time to time. Such samples must be refiltered and determined with the next set.)

If more than 0.5 mg. of selenium is present, dissolve it into a 100 ml. beaker, using 30 ml. of HBr containing 0.2–0.3 ml. of bromine and wash with 30 ml. of cold water. Precipitate as before and heat on the steam bath for 20 minutes, bringing the temperature to 45–50°C. (This second precipitation is necessary to free the selenium from sensible quantities of impurities.) Allow to stand 48 hours. Gather the selenium on an asbestos mat in a Gooch crucible and wash with cold water. Prepare a tare in a similar manner. Dry at 85–90°C. overnight, cool in a vacuum desiccator, and weigh against the tare.

SPECIAL TREATMENT WITH SULFURIC ACID

If the soil contains 0.1 p.p.m,. or less of selenium, use preferably the integration method (Robinson, *et al.*[1]). Add the distillate to a fresh charge of the soil with additional HBr and Br_2. (This procedure may be repeated until any desired amount of the sample has been used.) If the soil is calcareous, lateritic, or of high colloid content, add H_2SO_4 to the charge so that the water liberated in the reaction will not dilute the acid distillate and thereby fail to carry over the selenium. (A simple small hydrometer may be made for use in the receiving flask and calibrated with HBr and water.) If the distillate is less than 42% HBr, take a second distillate and increase the amount of H_2SO_4 correspondingly. If too much H_2SO_4 has been added and hydrogen bromide gas passes, as a fog, through the Br_2 water trap, add water through the thistle tube to correct this. In the regular distillation of lateritic soils, add H_2SO_4 to the charge to produce a distillate of 42% or stronger HBr.

SELENIUM IN SOIL RESIDUES

It has been shown by Robinson *et al.*,[2] that 0.002 mg. of selenium as sodium selenate when added to 1000 grams of soil can be recovered by distillation.

It is a natural supposition that pyrites, or other sulfides, would be decomposed during soil formation. It has been shown by Shorey[3] and confirmed in this laboratory, that after bromination of organic matter with a hydrobromic acid solution of bromine the selenium can be removed by distillation.

However, a test was made to find whether the selenium occurring naturally in soils can be removed completely by the usual method of distillation. Three samples of soil were selected: one from South Dakota, one from Kansas, and one from Colorado. After a second distillate had been taken, the residue in the distilling flask was poured into an excess of

[1] *Loc. cit.*
[2] *Loc. cit.*
[3] Private communication.

nitric acid. The bromine liberated was removed by gentle warming and 125 ml. of sulfuric acid was added. The mixture was boiled gently for 2 hours, and the remainder of the nitric acid was removed by evaporation on the steam bath. The sample was then distilled with hydrobromic acid and bromine and the distillate tested for selenium. The results are given in the table.

Selenium removed from different distillates

SOIL TYPE	FIRST DISTILLATE	SECOND DISTILLATE	DISTILLATE AFTER TREATMENT WITH $HNO_3-H_2SO_4$
	p.p.m. in soil	*p.p.m. in soil*	*p.p.m. in soil*
Boyd clay from S.D. Depth, 0–6″	3*		
	3	None	0.2
	3	0.02	0.6†
Niobrara clay loam from Kansas.	3*		
Depth, 0–6″	2	0.02	0.04
	3	None	
Orman clay loam from Colorado.	3*		
Depth, 0–8″	2.5	None	0.04
	2.5	None	0.4†

* Routine determination, and no further treatment of samples was made.
† 0.4 p.p.m. of selenium as Na_2SeO_3 was added to residue before treatment with $HNO_3-H_2SO_4$.

These results show that essentially all the selenium comes over in the first distillate. Treatment with nitric and sulfuric acids shows that the selenium is primarily present in forms readily removed by distillation with hydrobromic acid and bromine. Selenium added to the residue after regular distillation of the sample is completely recovered after treatment with nitric and sulfuric acids, showing that the treatment does not result in loss of selenium.

It is recommended[1] that the work be continued.

REPORT ON FERTILIZERS

By G. S. Fraps (Agricultural Experiment Station, College Station, Texas), *Referee*

The associate referees have continued their work, and as their recommendations will be given in their reports, it is not necessary to repeat them here.

It seems desirable to authorize the use of a factor weight for potash. If 2.422 grams is used instead of 2.5 grams, the solution made up to 200

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 52 (1937).

cc., and 50 cc. taken (equal to 0.4844 gram), it is only necessary to multiply the weight by 40 to get the percentage of potash. Corresponding figures are used for other quantities. This reduces the amount of calculation and avoids the necessity of referring to a table.

The barium chloride method for the determination of potash, *Methods of Analysis, A.O.A.C.*, 1935, 31, cannot be used for fertilizers, as the methods are now written, and if it is not needed in connection with other work, it should be removed from the methods.

After the residue for potash has been ignited and dissolved in water, insoluble material is sometimes present, especially if a quartz dish is used. Provision should be made for filtering at this point, if necessary.

The strength of the platinum chloride solution to be used for potash determinations is stated in the official method to be the equivalent of 1 gram of platinum in 10 cc. of solution. This would be equal to 4 grams of K_2O for 10 cc., or 0.4 gram for 1 cc., so that 1 cc. equals 80 per cent in 0.5 gram of substance. Even though an excess of platinum chloride is desirable, the solution is too strong. In this laboratory, a solution in which 1 cc. is equal to 1 per cent in 1 gram is used. It would be desirable to have the matter of a different platinum solution considered by the Associate Referee on Potash.

The A.O.A.C. methods contain no procedure for recovering platinum. It seems desirable to have a tentative method of this kind. In this laboratory, the platinum is reduced with pure aluminum in sticks. The platinum black is purified by boiling several times with hydrochloric acid, and the purified platinum is then dissolved in nitric and hydrochloric acids. The nitric acid is removed by evaporating with hydrochloric acid, until brucin gives no test for nitric acid. Platinum is estimated in the solution, and it is made up so that 1 cc. equals 1 per cent of potash in 1 gram of substance.

E. W. Magruder has called attention to the fact that some of the methods for total phosphoric acid in materials containing organic matter are not suitable for fertilizers containing cottonseed meal. This matter seems to require further study and the clear designation of suitable methods. It has also been stated that the kind of filter paper used affects the quantity of water-soluble phosphoric acid. This should be investigated.

The methods do not state whether the citrate-soluble phosphoric acid should be run soon after the water-soluble is washed out or whether the residue can be left overnight or longer without affecting the results. This should be looked into, and a definite statement made.

It has been said that some grades of reduced iron contain nitrogen and that the use of such iron in the reduced iron method gives too high results. This subject requires study.

H. A. Lepper calls attention to the fact that the new 1935 edition of *Methods of Analysis* contains an appendix giving directions for the preparation and standardization of sodium hydroxide and hydrochloric acid.

It is expected that this appendix will be enlarged to include other reagents, as well as other methods for standardization of acid based on direct gravimetric procedures. This, as Lepper states, brings up the question of the description of the directions for standardizing acids as given in the method for nitrogen in fertilizers, and it is possible these should be revised and included in the appendix. Changes in the methods for solutions used in fertilizer and other official work can be made only after all the active members having charge of the particular inspection affected have had opportunity to test the proposed changes, according to Article 6 of the By-laws.

The methods for the determination of nitrogen and protein now have a high degree of accuracy as shown by the results of the analyses of samples sent out by the American Oil Chemists Association and by the Fertilizer Chemists. This high degree of accuracy means that the methods of preparing the standard acid give highly agreeing results. For this reason no changes should be made hastily.

In some preliminary tests on phosphate rocks, it appears that some are acid instead of neutral, as required by the method for acid-forming or non-acid-forming quality of fertilizer. This work is only preliminary and may not be confirmed by subsequent work, but it seems to be a matter that should receive the attention of the Association.

The Referee wishes to commend the associate referees for the excellent work they are doing.

RECOMMENDATIONS[1]

These recommendations are supplementary to those made by the associate referees.

It is recommended—

(1) That the Associate Referee on Potash consider the matter of providing for the use of a factor weight, elimination of the barium chloride method, filtration of the potash solution after ignition and solution if necessary, changing the strength of the platinum chloride solution, and recommending a method for recovering platinum.

(2) That the Associate Referee on Phosphoric Acid consider the matter of designating the methods for total phosphoric acid that are suitable for fertilizers containing cottonseed meal, specifying the kind of filter paper suitable for estimation of water-soluble phosphoric acid, and stating how long the residue may wait between the process of washing out the water-soluble phosphoric acid and that of estimating the citrate-soluble phosphoric acid.

(3) That the Associate Referee on Nitrogen consider the matter of examining reduced iron to see if any lots contain nitrogen, elimination of the reduced iron method for nitrogen, and cooperation with the Referee

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 50 (1937).

on Standard Solutions in improving the methods for volumetric solutions used in the estimation of nitrogen.

(4) That the Associate Referee on Acid-forming or Non-acid-forming Quality of Fertilizer ascertain whether any samples of phosphate rocks are acid by the method for acid-forming or non-acid-forming quality in fertilizers.

REPORT ON PHOSPHORIC ACID

AVAILABILITY OF CALCINED PHOSPHATE AND OTHER NEW PHOSPHATIC MATERIALS AS DETERMINED BY CHEMICAL AND VEGETATIVE TESTS

By WM. H. ROSS, *Associate Referee*, and K. D. JACOB
(Division of Fertilizer Research, Bureau of Chemistry and Soils, Washington, D. C.)

At the 1931 meeting of this Association a report (*8*) was given on the composition and fertilizer efficiency of the insoluble phosphates in ammoniated superphosphates. This report described vegetative tests with Florida pebble and Tennessee brown-rock superphosphates that had been treated with quantities of free ammonia ranging from 2 to 8 per cent of the superphosphate. The fertilizer efficiencies of the ammoniated materials were determined by pot tests made in 15 State Experiment Stations and other institutions throughout the country. The same materials were also analyzed in 24 industrial and State laboratories by different modifications of the official method for determining phosphate availability.

In general, the availability of an ammoniated superphosphate to plants was shown to be greater by the pot tests than that indicated by the official method and therefore a recommendation was made that the method be changed to bring the results into closer agreement with those of vegetative tests. This recommendation was adopted at the 1931 meeting of this Association (*9*).

The present report describes a similar study that was made of several new phosphatic materials that give promise of being produced commercially for use as fertilizers. These materials are calcined phosphate, calcium metaphosphate, and fused phosphate rock.

COMPOSITION AND SOURCE OF PHOSPHATIC MATERIALS

The composition of the phosphates used in the investigation is given in Table 1.

Samples 1 and 2, mono- and dicalcium phosphates, are of C. P. grade. Sample 3 is a commercial superphosphate prepared from Tennessee brown-rock phosphate. Calcined phosphate (*1, 3, 5, 6*), Samples 4 to 10, inclusive, differ in their particle size. The material from which they were

TABLE 1—*Composition of phosphatic materials*

SAMPLE NO.	MATERIAL	FINENESS	PHOSPHORIC ACID (P_2O_5)			WEIGHT OF MATERIAL EQUIVALENT TO 1 GRAM OF TOTAL P_2O_5
			TOTAL	CITRATE-INSOLUBLE	CITRIC ACID-INSOLUBLE	
		mesh	*per cent*	*per cent*	*per cent*	*gram*
1	Monocalcium phosphate[a]	−40	56.76	0.00	0.00	1.762
2	Dicalcium phosphate	−40	48.53	2.77	0.00	2.061
3	Superphosphate, made from Tennessee brown rock[b]	−40	19.67	0.12	4.07	5.084
4	Calcined phosphate, cooled rapidly	−20	37.40	7.80	4.74	2.674
5	Calcined phosphate, cooled rapidly	−40	37.36	4.72	3.06	2.677
6	Calcined phosphate, cooled rapidly	−80	37.18	3.73	2.77	2.690
7	Calcined phosphate, cooled rapidly	−200	37.20	3.68	2.78	2.688
8	Calcined phosphate, cooled rapidly	20–40	37.30	9.53	5.29	2.681
9	Calcined phosphate, cooled rapidly	60–80	37.20	5.63	4.58	2.688
10	Calcined phosphate, cooled rapidly	100–150	37.05	4.46	3.44	2.699
11	Calcined phosphate, reverted	−80	37.05	20.50	13.87	2.699
12	Basic slag	−100	18.46	3.61	3.27	5.417
13	Calcium metaphosphate	−80	63.87	0.24	43.83	1.566
14	Fused phosphate rock	−80	28.97	4.60	2.49	3.452
15	"Non-acid" phosphate[c]	−100	26.56	11.40	9.68	3.765
16	Tennessee brown-rock phosphate	−100	33.75	31.37	27.87	2.963

(a) 54.44% of water-soluble P_2O_5.
(b) 12.74% of water-soluble P_2O_5.
(c) 2.71% of water-soluble K_2O, equivalent to 0.102 gram of water-soluble K_2O per gram of total P_2O_5.

prepared was produced experimentally by calcining Tennessee brown-rock phosphate in a rotary kiln at 1400°C. in the presence of water vapor. The product obtained in this way was thoroughly mixed, ground, and screened to give the desired range of particle size. Calcined phosphate resembles basic slag in many of its properties. Experience has shown that basic slag gives best results when it is finely ground. The object in preparing samples of calcined phosphate of varying particle size was to determine if fine grinding increases the fertilizer efficiency of this material as in the

TABLE 2.—*Mechanical and chemical compositions of calcined phosphate samples*

MECHANICAL COMPOSITION								
SAMPLE NO.	FINENESS	MECHANICAL COMPOSITION, MESH						
		20–40	40–60	60–80	80–100	100–150	150–200	–200
	mesh	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	–20	50.2	24.8	7.7	4.3	4.5	3.3	5.2
5	–40	—	25.7	17.3	10.5	11.3	9.7	25.5
6	–80	—	—	—	9.7	22.5	20.0	47.8

CHEMICAL COMPOSITION					
SAMPLE NO.	FINENESS	TOTAL P_2O_5	FLUORINE	INSOLUBLE P_2O_5 IN SAMPLES REGROUND TO 200-MESH	
				CITRATE-INSOLUBLE P_2O_5	CITRIC ACID-INSOLUBLE P_2O_5
	mesh	*per cent*	*per cent*	*per cent*	*per cent*
7	–200	37.20	0.09	3.68	2.78
8	20–40	37.30	0.05	2.64	1.82
9	60–80	37.20	0.15	4.92	4.36
10	100–150	37.05	0.13	4.29	3.56

case of basic slag. That the particle size of calcined phosphate has a marked effect on its citrate-solubility has recently been shown by Jacob, Rader and Tremearne (*2*).

The mechanical compositions of calcined phosphate, Samples 4, 5 and 6, and the chemical compositions of Samples 7, 8, 9 and 10 are given in Table 2.

A calcined phosphate that has been cooled quickly from the calcination temperature of 1400° C. to room temperature is usually much more soluble in neutral ammonium citrate solution than one that has been cooled slowly through this temperature range (*4*). Sample 11 was prepared by heating Sample 6 to 1000°C. and maintaining it at this temperature for one hour. This treatment greatly reduced the citrate-solubility of the material without producing any appreciable change in its total P_2O_5 content.

Sample 12 is an imported basic slag. It was considered advisable to

include this material in the series with calcined phosphate in order that a direct comparison might be made of the fertilizer efficiencies of the two materials.

Calcium metaphosphate Sample 13 was prepared on a pilot plant scale by the Tennessee Valley Authority at Muscle Shoals by treating Tennessee brown-rock phosphate at a temperature of 1000°–1100°C. with the P_2O_5 fumes evolved in the smelting of phosphate rock in an electric furnace. The fused phosphate rock, Sample 14, also made experimentally by the Tennessee Valley Authority, was prepared by blowing wet air through fused Tennessee brown-rock phosphate. The chemical reactions involved in the preparation of this material and the chemical and physical properties of the product seem to be the same as those for calcined phosphate. "Non-acid" phosphate Sample 15 is the trade name of a commercial product formerly manufactured in this country by heating a mixture of Florida pebble phosphate rock and a potash salt in a rotary kiln.

Sample 16 is a commercial grade of ground Tennessee brown-rock phosphate. Its mechanical analysis is as follows:

	per cent
Through 100-mesh—	100
Through 200-mesh—	98
Through 300-mesh—	92
Through 400-mesh—	86

DIRECTIONS FOR POT TESTS

In submitting these samples for vegetative tests it was suggested that all samples be used on the basis of their total P_2O_5 content without regard to their citrate- or citric acid-solubility and that the following information be given on the details of the pot tests:

1. Number of replications.
2. Soil type.
3. *p*H of soil.
4. If soil has been limed recently, state length of time elapsing between liming and application of fertilizer for pot tests.
5. Weight of air-dry soil per pot.
6. P_2O_5 application expressed in terms of grams of P_2O_5 per pot and pounds of P_2O_5 per acre.
7. Nitrogen fertilizer, kind and pounds of N per acre.
8. Potash fertilizer, kind and pounds of K_2O per acre.
9. Method of applying fertilizer. State whether the phosphate was uniformly distributed throughout the entire mass of soil or was applied in a band. If it was applied in a band, state the thickness of the band and its approximate position with respect to the seed or plants. State whether the N and K_2O were applied entirely at the time of planting, or partially at planting and partially as top-dressing at a later date.
10. Kind of crop grown.
11. Date of seeding or transplanting.
12. Number of plants per pot.

TABLE 3.—*Relative availability of phosphatic materials as shown by pot tests with soils having a pH of 6.00 or below*

PHOSPHATIC MATERIAL	FINENESS	RESPONSE TO PHOSPHATE FERTILIZATION ON BASIS OF MONOCALCIUM PHOSPHATE=100											
		BARTHOLOMEW ARKANSAS SUDAN GRASS[1]		COOK MICHIGAN SUDAN GRASS[2]		EDWARDS HAWAII SUDAN GRASS[3]		DE TURK ILLINOIS AV. DRY WT. OF CROP[4]		DE TURK ILLINOIS AV. DRY WT. OF CROP[5]		HOAGLAND CALIFORNIA TOMATO[6]	
		AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	WHEAT	SWEET CLOVER	WHEAT	SWEET CLOVER	AV. DRY WT. OF CROP	RELATIVE INCREASE
	mesh	*grams*	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
1 Monocal. phos.	−40	23.4	100	23.8	100	152.1	100	—	—	—	—	46.5	100
2 Dical. phos.	−40	24.9	144	24.7	111	188.8	203	—	—	—	—	54.9	118
3 Superphos.	−40	24.2	124	23.7	99	201.1	237	21.9	4.1	23.7	24.2	—	—
4 Calc. phos., cooled rapidly	−20	21.9	56	22.8	88	193.6	216	14.3	7.1	23.3	23.6	—	—
5 " "	−40	24.0	118	24.5	109	197.8	228	—	—	—	—	—	—
6 " "	−80	24.0	118	22.2	80	194.2	218	—	—	—	—	55.9	120
7 " "	−200	25.5	162	21.2	68	184.9	191	—	—	—	—	—	—
8 " "	20–40	23.9	115	17.9	27	173.8	161	—	—	—	—	42.3	91
9 " "	60–80	21.9	56	23.9	101	189.7	205	16.2	4.4	23.8	27.4	57.3	123
10 " "	100–150	23.4	100	22.8	88	187.3	198	—	—	—	—	—	—
11 Calc. phos., rev.	−80	22.4	71	22.0	78	182.2	184	—	—	—	—	—	—
12 Basic slag	−100	23.4	100	19.6	48	189.7	206	—	—	—	—	56.2	121
13 Cal. metaphos.	−80	22.5	74	22.7	86	173.2	159	21.6	4.2	22.8	25.5	56.8	122
14 Fused phos. rock	−80	23.3	97	21.4	70	187.6	199	23.0	7.8	25.1	26.5	54.1	116
15 "Non-acid" phos.	−100	23.6	106	21.9	77	173.1	159	—	—	—	—	—	—
16 Tenn. rock phos.	−100	20.9	26	19.8	51	148.6	90	9.0	4.6	23.1	22.5	—	—
No phos.		20.0		15.7		116.4		4.4	0.1	19.8	14.9	0.2	

TABLE 3.—*Continued*

PHOSPHATIC MATERIAL	FINENESS	RESPONSE TO PHOSPHATE FERTILIZATION ON BASIS OF MONOCALCIUM PHOSPHATE = 100														
		MORGAN CONNECTICUT TOBACCO[7]			PEMBER RHODE ISLAND JAP. MILLET[8]		PIERRE WEST VIRGINIA SUDAN GRASS[9]		REID DEPT. OF AGR. GERMAN MILLET[10]		THOMAS MARYLAND WHEAT[11]		TIDMORE ALABAMA SORGHUM[12]		AV. RELATIVE INCREASE
		AV. DRY WT. OF CROP	RELATIVE INCREASE	UNIVERSAL SOIL TEST	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	
	mesh	*grams*	*per cent*	*lbs. per acre*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*pr cent*	*grams*	*per cent*	*grams*	*per cent*	*per cent*
1 Monocal. phos.	−40	7.21	100	83	39.5	100	22.6	100	20.47	100	7.5	100	1.6	100	100
2 Dical. phos.	−40	8.42	118	100	—	—	23.2	106	24.97	158	8.0	114	1.5	91	129
3 Superphos.	−40	1.45	13	70	—	—	22.7	101	22.69	129	5.0	29	2.9	218	116
4 Calc. phos., cooled rapidly	−20	—	—	—	39.8	105	21.8	92	18.77	78	7.0	86	3.0	227	119
5 " "	−40	—	—	—	—	—	23.5	109	21.34	111	7.0	86	3.4	264	146
6 " "	−80	8.85	125	112	41.0	123	25.0	124	21.57	114	6.5	71	3.2	245	134
7 " "	−200	—	—	—	42.0	138	24.5	119	23.44	138	7.5	100	2.3	164	135
8 " "	20–40	2.00	22	36	37.8	74	18.6	60	17.99	68	5.5	43	2.5	182	85
9 " "	60–80	2.79	33	77	39.0	92	24.6	120	25.12	160	6.5	71	6.2	518*	107
10 " "	100–150	5.74	78	117	40.5	115	23.3	107	25.99	171	6.5	71	6.2	518*	116
11 Calc. phos., rev.	−80	4.33	57	43	39.5	100	25.2	126	20.98	107	6.0	57	2.9	218	111
12 Basic slag	−100	3.50	44	87	—	—	24.0	114	21.07	108	7.0	86	2.3	164	110
13 Cal. metaphos.	−80	1.50	14	54	38.0	77	25.1	125	24.42	151	8.5	129	1.9	127	106
14 Fused phos. rock	−80	5.65	76	114	—	—	25.7	131	25.85	170	6.0	57	2.4	173	121
15 "Non-acid" phos.	−100	—	—	—	—	—	23.7	111	25.01	159	6.5	71	1.2	64	107
16 Tenn. rock phos.	−100	0.15	−6	24	—	—	17.8	52	10.55	−28	4.5	14	0.8	27	28
No phos.		0.58		15	33.0		12.6		12.74		4.0		0.5		

* Not included in average.

Continued on opposite page

13. Date of harvesting.
14. Dry weight of harvested crop.

The study that was made of ammoniated superphosphates in 1931 indicated that the heavily ammoniated products were more effective in promoting plant growth when used on soils having a *p*H below 6.0 than on alkaline or nearly neutral soils. It was accordingly suggested that the pot tests with calcined phosphates be made on soils having *p*H values below and above 6.0, as well as on soils of low and of high phosphate-fixing power.

The following collaborators submitted reports:

1. Barnes, Earl E., Ohio Agr. Expt. Station, Wooster, Ohio.
2. Bartholomew, R. P., Arkansas Agr. Expt. Station, Fayetteville, Ark.
3. Cook, R. L., Michigan State College, East Lansing, Mich.
4. DeTurk, E. E., Illinois Agr. Expt. Station, Urbana, Ill.
5. Edwards, Dorsey W., Hawaii Agr. Expt. Station, Honolulu, Hawaii.
6. Hoagland, D. R., Univ. of California, Berkeley, Calif.
7. Magnuson, H. P., Idaho Agr. Expt. Station, Moscow, Idaho.
8. McGeorge, W. T., Arizona Agr. Expt. Station, Tucson, Ariz.
9. Morgan, M. F., Connecticut Agr. Expt. Station, New Haven, Conn.
10. Myers, H. E., Kansas State College, Manhattan, Kans.
11. Pember, F. R., Rhode Island Agr. Expt. Station, Kingston, R. I.
12. Pierre, W. H., West Virginia Agr. Expt. Station, Morgantown, W. Va.
13. Reid, Fred R., Bureau of Plant Industry, U. S. Dept. Agr., Washington, D. C.
14. Thomas, R. P., Maryland Agr. Expt. Station, College Park, Md.
15. Tidmore, J. W., Alabama Agr. Expt. Station, Auburn, Ala.

The results obtained on soils having a *p*H of 6.00 or below are given in Table 3, and those obtained on soils having a *p*H above 6.0 are given in

TABLE 3.—*Continued*

[1] Grown in triplicate on Clarksville silt loam soil, *p*H = 5 42. Each pot of 9 kg of soil received the equivalent of 60 lbs. of P_2O_5, 21.5 lbs. of N as $(NH_4)_2SO_4$, 16 lbs. of N as $NaNO_3$, and 125 lbs. of K_2O as KCl per acre

[2] Grown in quadruplicate on Miami soil, *p*H = 5.3. Each pot of 4 kg. of soil received the equivalent of 80 lbs. of P_2O_5, 20 lbs. of N, and 20 lbs of K_2O per acre.

[3] Grown in duplicate on a dark reddish brown clay soil, *p*H = 5 2 Each pot of 5 kg of soil received the equivalent of 1300 lbs. of P_2O_5, 318 lbs of N as NH_4NO_3, and 433 lbs. of K_2O as K_2SO_4 per acre

[4] Grown in duplicate on Clinton silt loam soil, *p*H = 4.4 Each pot of 8.3 kg of soil received the equivalent of 126 lbs. of P_2O_5 per acre without N or K_2O.

[5] Grown in duplicate on Muscatine silt loam soil, *p*H = 5.4. Each pot of 6 8 kg of soil received the equivalent of 126 lbs. of P_2O_5 per acre without N or K_2O.

[6] Grown in sextuplicate on virgin Aiken clay soil, *p*H = 6.00. Each pot of 8 kg. received the equivalent of 2.0 g. of P_2O_5 per pot and 0.7 g. of N as $Ca(NO_3)_2$.

[7] Grown in duplicate on Melrose loamy fine sand, *p*H = 5.00. Each pot of 4 kg of soil received the equivalent of 0.5 g. of P_2O_5, 0 3 g of nitrate N, 0.2 g of ammonia N, 1.0 g of K_2O, and 0 3 g. of MgO. At midgrowth all treatments received 0 5 g. of N as calurea.

[8] Grown in duplicate on limed Bridgehampton very fine sandy loam soil, *p*H = 5.5. Each pot of 6.5 kg. of soil received, at time of planting, the equivalent of 1 g of P_2O_5, 0.165 g. of N as $NaNO_3$, and 1.08 g of K_2O as K_2SO_4; and later two applications of 1 g. of $NaNO_3$ in 100 cc. of water.

[9] Grown in triplicate on DeKalb silty loam soil, *p*H = 5.33. Each pot of 7 kg. of soil received the equivalent of 100 lbs. of P_2O_5, 100 lbs of N at planting time, 20 lbs of N later as a top dressing and 126 lbs of K_2O as KCl per acre. The N was applied as $NaNO_3$ and urea in equal parts

[10] Grown in triplicate on Norfolk loamy fine sand soil, *p*H = 5 8 Each pot of 5 kg. of soil received the equivalent of 240 lbs. of P_2O_5, 33.3 lbs. of N as $NaNO_3$, 66.6 lbs. of N as $(NH_4)_2SO_4$, and 120 lbs. of K_2O as K_2SO_4 per acre.

[11] Grown in duplicate on Chester loam soil, *p*H = 5 6. Each pot of 4 kg. received the equivalent of 120 lbs. of P_2O_5, 40 lbs. of N as $(NH_4)_2SO_4$, and 60 lbs. of K_2O as KCl per acre.

[12] Grown in triplicate on Cecil clay soil, *p*H = 4.8. Each pot of 8 kg. of soil received the equivalent of 120 lbs. of P_2O_5, 50 lbs. of N, and 50 lbs. of K_2O per acre. The P_2O_5 was mixed with upper one-half of soil; N and K_2O was applied as top dressing.

TABLE 4.—*Relative availability of phosphatic materials as shown by pot tests with soils having a pH above 6.00*

PHOSPHATIC MATERIAL	FINENESS	RESPONSE TO PHOSPHATE FERTILIZATION ON BASIS OF MONOCALCIUM PHOSPHATE=100											
		BARNES OHIO SUDAN GRASS[1]		BARTHOLOMEW ARKANSAS SUDAN GRASS[2]		DE TURK ILLINOIS AV. DRY WT. OF CROP[3]		MC GEORGE ARIZONA TOMATO[4]		PEMBER RHODE ISLAND JAP. MILLET[5]		PIERRE WEST VIRGINIA SUDAN GRASS[6]	
		AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	WHEAT	SWEET CLOVER	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE	AV. DRY WT. OF CROP	RELATIVE INCREASE
	mesh	*grams*	*per cent*	*grams*	*per cent*	*grams*	*grams*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*per cent*
1 Monocal. phos.	−40	4.6	100	19.4	100	—	—	11.00	100	56.5	100	21.0	100
2 Dical. phos.	−40	4.8	129	20.9	118	—	—	7.10	43	—	—	23.1	115
3 Superphos.	−40	—	—	20.0	107	23.6	26.4	9.30	75	—	—	22.8	113
4 Calc. phos., cooled rapidly	−20	—	—	18.0	83	20.2	28.0	8.43	63	51.0	80	20.4	96
5 " "	−40	—	—	18.0	83	—	—	7.60	50	—	—	24.4	124
6 " "	−80	4.7	114	21.1	120	—	—	7.82	54	52.3	85	23.4	117
7 " "	−200	—	—	20.0	107	—	—	9.79	82	50.0	77	23.7	119
8 " "	20–40	4.6	100	17.7	80	—	—	8.15	58	46.8	65	18.3	81
9 " "	60–80	5.5	229	18.9	94	21.4	26.1	5.92	26	53.0	87	21.2	101
10 " "	100–150	—	—	20.7	116	—	—	4.80	10	57.0	102	24.5	124
11 Calc. phos., rev.	−80	—	—	20.7	116	—	—	5.37	18	47.8	69	20.8	99
12 Basic slag	−100	6.1	314	21.7	128	—	—	6.50	34	—	—	22.4	110
13 Calc. metaphos.	−80	8.1	600	21.8	129	21.8	23.6	6.65	37	55.0	95	24.5	124
14 Fused phos. rock	−80	6.1	314	21.3	123	23.4	30.3	5.38	18	—	—	24.2	122
15 "Non-acid" phos.	−100	—	—	20.8	117	—	—	7.70	52	—	—	21.9	106
16 Tenn. rock phos.	−100	—	—	16.6	66	15.8	17.8	4.00	−2	—	—	10.2	25
No phos.		3.9		11.1		12.3	12.2	4.14		28.8		6.6	

Continued on opposite page.

Table 4. The soils used by Magnuson and by Myers, one of the soils used by Barnes, and one of those used by Cook showed little or no response to phosphate fertilization in these tests, and the results obtained therewith are not included in the tables. Results obtained by DeTurk showing the crop response that occurred with increased application of superphosphate Sample 3, calcined phosphate Sample 4, and with calcium metaphosphate Sample 13 are given in Table 5.

DISCUSSION OF AGRONOMIC RESULTS

The results reported by the collaborators are not so consistent as would be desired, but the following conclusions appear to be indicated:

1. On acid soils the fertilizer efficiency of the P_2O_5 in the finely ground calcined phosphate, calcium metaphosphate, and fused phosphate rock used in this collaborative study is similar to that of the P_2O_5 in monocalcium phosphate and superphosphate.
2. The calcined phosphate, calcium metaphosphate, and fused phosphate rock used in this collaborative study are just as effective in promoting plant growth on soils having *p*H values up to at least 7.14 as on soils having *p*H values below 6.0. The results obtained with the calcareous soil of *p*H 9.5 (McGeorge, Arizona Agr. Expt. Station) indicate a lower availability of these phosphates on soils of this type.
3. The response of crops to increased applications of calcined phosphate or calcium metaphosphate is similar to that produced by corresponding applications of superphosphate.
4. Calcined phosphate of 40 mesh or coarser is not so readily available to plants as is the more finely divided material.
5. Calcined phosphate that has been reverted by slow cooling is not so readily available to plants as is the rapidly cooled, unreverted product of the same degree of fineness.

AVAILABILITY OF CALCINED PHOSPHATE AND OTHER PHOSPHATES BY CHEMICAL TESTS

According to the present official method of analysis, the availability of all phosphate fertilizers, except basic slag, is determined by means of the neutral ammonium citrate method. The availability of basic slag is determined by the 2 per cent citric acid method. Calcined phosphate resembles basic slag in many of its properties, and the question has been raised as to whether the ammonium citrate method or the citric acid

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TABLE 4.—*Continued*

[1] Grown in triplicate on Wooster silt loam soil, *p*H = 6.02. Each pot of 1 gal. received the equivalent of 60 lbs. of P_2O_5, 45 lbs. of N as KNO_3 and urea, and 45 lbs. of K_2O as KNO_3 per acre.

[2] Grown in triplicate on Clarksville silt loam soil, *p*H = 7.14. Each pot of 9 kg. of soil received the equivalent of 60 lbs. of P_2O_5, 21.5 lbs. of N as $(NH_4)_2SO_4$, 16 lbs. of N as $NaNO_3$, and 125 lbs. of K_2O as KCl per acre.

[3] Grown in duplicate on limed Muscatine silt loam soil, *p*H = 6.4. Each pot of 6.8 kg. of soil received the equivalent of 126 lbs. of P_2O_5 per acre without N or K_2O.

[4] Grown in duplicate on a calcareous sandy loam soil, *p*H = 9.5. Each pot of 3 kg of soil received the equivalent of 0.5 g. of P_2O_5 without N or K_2O.

[5] Grown in duplicate on Bridgehampton very fine sandy loam soil, *p*H = 6.15. Each pot of 6.2 kg of soil received, at time of planting, the equivalent of 1 g of P_2O_5, 0.165 g of N as $NaNO_3$, and 1.08 g. of K_2O as K_2SO_4; and later two applications of 1 g. of $NaNO_3$ in 100 cc. of water.

[6] Grown in triplicate on DeKalb silty loam soil, *p*H = 6.13. Each pot of 7 kg. of soil received the equivalent of 100 lbs. of P_2O_5, 100 lbs. of N at planting time, 20 lbs. later as a top dressing, and 126 lbs of K_2O as KCl per acre. The N was applied as $NaNO_3$ and urea in equal parts.

TABLE 5.—*Crop response to increased applications of phosphate*

P_2O_5 ADDED	DETURK ILLINOIS					
	CLINTON SILT LOAM[1] AV. DRY WT. OF CROP		MUSCATINE SILT LOAM[2] AV. DRY WT. OF CROP		MUSCATINE SILT LOAM[3] AV. DRY WT. OF CROP	
	WHEAT	SWEET CLOVER	WHEAT	SWEET CLOVER	WHEAT	SWEET CLOVER
lbs. per acre	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
			Superphosphate Sample 3			
12.6	8.0	0.6	21.9	17.2	17.8	17.4
40.0	20.0	2.5	23.6	19.9	19.5	23.6
126.0	21.9	4.1	23.7	24.2	23.6	26.4
			Calcined phosphate Sample 4			
12.6	6.5	0.8	20.9	19.1	18.1	18.5
40.0	10.9	2.6	20.8	20.3	20.1	25.1
126.0	14.3	7.1	23.3	23.6	20.2	28.0
225.0	19.4	7.8	22.9	25.9	22.6	29.6
400.0	18.9	8.6	25.5	31.2	23.5	30.4
			Calcium metaphosphate Sample 13			
0.0	4.4	0.1	19.8	14.9	12.3	12.2
12.6	6.3	0.5	21.1	14.4	15.1	15.1
22.5	7.9	1.4	21.4	17.9	18.0	19.9
40.0	11.1	2.4	22.0	17.7	16.7	21.1
71.1	15.7	2.1	22.8	18.9	20.6	18.6
126.0	21.6	4.2	22.8	25.5	21.8	23.6
225.0	23.7	7.0	27.0	30.5	24.6	30.0
400.0	25.3	7.7	27.8	24.8	24.2	27.7

[1] pH = 4.4.
[2] pH = 5.4.
[3] pH = 6.4.

method is better adapted to the evaluation of calcined phosphate. A collaborative study of the availability of calcined phosphate as determined both by the ammonium citrate and citric acid methods was accordingly undertaken with a view to determining which method gives results in better agreement with the vegetative tests. Calcium metaphosphate Sample 13, fused phosphate rock Sample 14 and a heavily ammoniated superphosphate Sample 16 were also included in this collaborative study.

The samples submitted to each of the collaborators were as follows:

Sample No.

5 Calcined phosphate, cooled rapidly, through 40 mesh.
6 Calcined phosphate, cooled rapidly, through 80 mesh.
8 Calcined phosphate, cooled rapidly, 20–40 mesh.
11 Calcined phosphate, cooled slowly, through 80 mesh.
12 Basic slag.
13 Calcium metaphosphate.
14 Fused phosphate rock.
17 Ammoniated superphosphate.

DIRECTIONS FOR ANALYSIS

A.—Determine citrate-insoluble P_2O_5 as follows in all samples, except Sample 17, without previous washing, and in the residue of Sample 17 when washed as directed in *Methods of Analysis, A.O.A.C.*, 1935, 21, **13**.

Heat 100 cc. of the ammonium citrate solution to 65°C. in a 250 cc. flask placed in a water bath, keeping the flask loosely stoppered to prevent evaporation. Keep the level of the water in the bath above that of the citrate solution in the flask. When the temperature of the citrate solution has reached 65°C., drop into it a 9 cm. filter paper containing 1 gram of the sample (or the washed residue from 1 gram in the case of Sample 17). Close the flask tightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, releasing the pressure by momentarily removing the stopper. Return the flask to the bath and maintain its contents at exactly 65°C. Shake the flask for approximately 5 seconds at 5 minute intervals. At the expiration of 1 hour from the time the filter paper and residue were introduced, remove the flask from the bath and immediately filter the contents with suction through a No. 5 Whatman filter, or other filter of equal retentiveness, using a Büchner or ordinary glass funnel with platinum or other cone, and wash with water at 65°C. until the volume of the filtrate is about 350 cc. If water gives a cloudy filtrate, wash with a 5% solution of NH_4NO_3. Transfer the wet filter with contents to a 200 cc. flask, add 30–35 cc. of HNO_3 and 5–10 cc. of HCl, and boil until all phosphate is dissolved. Dilute the solution to 200 cc., mix well, filter through a dry filter, and proceed as directed in *Methods of Analysis, A.O.A.C.*, 1935, 20–21, 9 or **12(a)**.

B.—Determine citrate-insoluble P_2O_5 in Samples 6, 11 and 12 as directed under *A*, with the single variation that the weighed unwashed sample be transferred directly into the digestion flask without the use of a filter paper.

(In a paper presented at the last meeting of the A.O.A.C., Jacob, Rader, and Tremearne (*2*) showed that lower results are obtained for citrate-insoluble P_2O_5 in calcined phosphate and similar materials when filter paper is present at the time that the sample is digested in the citrate solution than when it is not present. The determination of citrate-insoluble P_2O_5 in Samples 6, 11 and 12 without the use of a filter paper in the citrate solution is recommended in order to give official recognition to this observation.)

C.—Determine citric acid-insoluble P_2O_5 in each of the standard samples as follows:

Weigh 5 grams of the sample into a 500 cc. Wagner flask containing 5 cc. of 95% alcohol. (The neck of the flask should have a width of at least 22 mm. and the graduation marks should be at least 8 cm. below the mouth.) Make up to the mark with 2% citric acid solution of a temperature of 17.5°C. Fit the flask with a rubber stopper, place at once in an end-over-end shaking apparatus, and shake the flask for 30 min. at the rate of 30–40 r.p.m. (*Methods of Analysis, A.O.A.C.*, 36, **62**). Remove the flask and immediately filter the contents with suction through a No. 5 Whatman filter, or other filter of equal retentiveness, using a Büchner or ordinary glass funnel with platinum or other cone, and wash with about 200 cc. of water at room temperature. Transfer the wet filter with contents to a 200 cc. flask, add 30–35 cc. of HNO_3 and 5–10 cc. of HCl, and boil until all phosphate is dissolved. Dilute the solution to 200 cc., mix well, filter through a dry filter, and proceed as directed in *Methods of Analysis, A.O.A.C.*, 20–21, 9 or **12(a)**. (If an end-over-end shaking apparatus is not available, treat 2 grams of the sample with 2 cc. of alcohol and 198 cc. of 2% citric acid, and stir for 30 min. in a stirring machine.)

D.—If the volumetric method is used in the determination of citrate-insoluble P_2O_5 in Samples 8 and 11, withdraw a second aliquot part from the filtered solution, prepared as directed under *A*, and proceed as directed in *Methods of Analysis*,

A.O.A.C., 21, **12(a)**, with the variation that the use of the phenolphthalein indicator be replaced by the addition of 15 cc. of a solution prepared by dissolving 0.015 gram of phenolphthalein in 100 cc. of U.S.P. formaldehyde and neutralizing with NaOH or KOH. When using this modification of the official method, prepare the standard NaOH or KOH solution, *Methods of Analysis, A.O.A.C.*, 20, **10(b)**, by diluting 366.2 cc. of normal alkali, free from carbonates, to 1 liter, or use the official standard alkali solution and multiply the number of cc. used in the titration by the factor 0.8842, in order that 1 cc. may be equivalent to 1% of P_2O_5 on a basis of 0.1 gram of substance.

(This modification of the official volumetric method was suggested by P. McG. Shuey, who states that the use of formaldehyde increases the sharpness of the end point in the titration, particularly in the analysis of samples that contain a relatively high percentage of P_2O_5. It has been shown that the presence of formaldehyde involves a second reaction, which requires the addition of about 5 cc. of 0.3238 *N* alkali above the needs of the first reaction, thus making necessary the use of a factor when titrating with the official standard alkali solution as referred to above.)

E.—Determine citrate-insoluble P_2O_5 in Samples 6, 11, and 17 as described under *A* with the single variation that filtration is made with suction through a Shimer filter with a mat of filter paper in place of a No. 5 Whatman paper on a Büchner or ordinary glass funnel.

(The filter pulp for the Shimer filter is most conveniently prepared from Schleicher and Schüll's No. 292 filter pulp disks. When these are not available, prepare the filter pulp by tearing from five to eight 9 cm. filters, or the equivalent of sheet filters, into shreds, place the pieces in a 500 cc. Erlenmeyer flask, add 250 cc. of hot water, close the flask with a rubber stopper, and, under the protection of a towel, shake vigorously until the paper is reduced to a pulp. The mat in the Shimer filter should be ¼–⅜″ thick when compacted by suction. It is necessary to compact the filter mat by suction in order to make it sufficiently retentive to hold the residue, and to prevent disruption of its upper surface when the solution to be filtered is poured directly on the filter mat. A rubber stopper fastened to a glass rod may be used to compact the filter.)

NOTES

1. The phosphorus in the calcium metaphosphate sample must be converted into the orthophosphate before it can be precipitated by the molybdate solution. In order to insure that this change has taken place, dissolve the ammonium citrate- and citric acid-insoluble residues from Sample 3 (see *A* and *C*, respectively) in 30–35 cc. of HNO_3 and 3–5 cc. of HCl, evaporate the resulting solution on a water bath, take up in a dilute solution of HNO_3, and boil for a few minutes.

2. It is suggested that all directions for making the citrate- and citric acid-insoluble determinations be followed as closely as possible.

3. It is possible that some of the materials may segregate to some extent on handling, and it is therefore recommended that all samples be thoroughly mixed before a portion is taken for analysis.

4. It is very important that the ammonium citrate solution used in this work be exactly neutral as determined by a *p*H comparator set.

5. At the last meeting of the A.O.A.C. a recommendation was adopted that a further study be made of the application of filter pulp in the Shimer filter to the filtration of citrate-insoluble residues. Direction *E* is included in compliance with this recommendation.

ANALYTICAL RESULTS

Reports were received from the following analysts who collaborated in this work:

1. Allen, H. R. and Gault, Lelah, Univ. of Kentucky, Lexington, Ky.
2. Austin, W. R., Armour Fertilizer Works, Nashville, Tenn.
3. Butt, C. A. and Hammett, A. M., International Agricultural Corpn., East Point, Ga.
4. Byers, C. R., Armour Fertilizer Works, Carteret, N. J.
5. Caldwell, R. D., Armour Fertilizer Works, Atlanta, Ga.
6. Charlton, R. C., American Agricultural Chemical Co., Baltimore, Md.
7. Cowan, E. W., Univ. of Missouri, Columbia, Mo.
8. Dupont, L. E., International Agricultural Corpn., Columbia, Tenn.
9. Ford, O. W., Indiana Agr. Expt. Station, Lafayette, Ind.
10. Hardin, L. J., Tennessee Agr. Expt. Station, Knoxville, Tenn.
11. Howes, C. Clifton, Davison Chemical Corpn., Baltimore, Md.
12. Janes, Egbert, International Agricultural Corpn., Mulberry, Fla.
13. Jones, R. L., Armour Fertilizer Works, Chicago Heights, Ill.
14. Koch, R. C., Swift and Company Fertilizer Works, Chicago, Ill.
15. Lamb, R. H., Darling and Co., East St. Louis, Ill.
16. Montague, H. S., Mississippi State College, State College, Miss.
17. Neu, Rudolph, Armour Fertilizer Works, Jacksonville, Fla.
18. Nichol, W. E., Seed, Feed and Fertilizer Laboratories, Ottawa, Can.
19. Potvin, Alfred, Seed, Feed and Fertilizer Laboratories, Ottawa, Can.
20. Rader, Jr., L. F., Bureau of Chemistry and Soils, Washington, D. C.
21. Ryder, W. A., F. S. Royster Guano Co., Norfolk, Va.
22. Shuey, P. McG., Shuey and Company, Savannah, Ga.

The results submitted by the collaborators are given in Tables 6–9.

DISCUSSION OF ANALYTICAL RESULTS

1. The results reported for insoluble P_2O_5 in most of the samples analyzed varied greatly, particularly in the case of citrate-insoluble P_2O_5 in Samples 5 and 8, and citric acid-insoluble P_2O_5 in Samples 5, 8, and 13. Nearly all the collaborators reported disagreement in their duplicate determinations, and some of the results given in Table 6 represent the mean of as many as ten determinations.

TABLE 6.—*Per cent citrate-insoluble and citric acid-insoluble P_2O_5 in standard phosphate samples*

COLLABORATOR	CALCINED PHOSPHATE, COOLED RAPIDLY −40 MESH	−80 MESH	20–40 MESH	REVERTED CALCINED PHOSPHATE	BASIC SLAG	CALCIUM META-PHOSPHATE	FUSED PHOSPHATE ROCK	AMMONIATED SUPER-PHOSPHATE
			Citrate-insoluble P_2O_5 (Direction A)					
1	5.20	3.65	14.55	20.85	3.45	0.15	4.30	5.05
2	5.65	3.10	10.15	20.55	3.05	0.38	4.17	4.35
3	3.80	3.17	6.90	20.12	3.43	0.07	4.34	4.10
4	4.90	3.20	10.90	20.37	3.45	0.20	4.80	3.80
5	5.38	3.27	10.02	20.13	4.02	0.14	4.55	4.75
6	3.86	2.93	8.33	19.73	3.26	0.10	3.87	3.57

TABLE 6.—*Continued*

Citric acid-insoluble P_2O_5 (Direction C)

COLLABORATOR	CALCINED PHOSPHATE, COOLED RAPIDLY −40 MESH	−80 MESH	20–40 MESH	REVERTED CALCINED PHOSPHATE	BASIC SLAG	CALCIUM META-PHOSPHATE	FUSED PHOSPHATE ROCK	AMMONIATED SUPER-PHOSPHATE
7	5.04	3.06	9.49	20.33	3.10	0.24	4.57	5.40
8	4.36	3.20	9.04	20.37	3.53	0.00	5.05	5.51
9	4.31	2.95	10.88	19.74	3.40	0.18	3.77	4.10
10	3.90	3.13	9.55	20.38	3.88	trace	4.45	4.55
11	5.02	3.04	10.66	20.87	3.57	0.40	4.56	3.90
12	4.67	3.32	13.50	21.21	3.39	0.27	4.24	4.88
13	6.55	4.10	17.75	21.80	4.00	0.40	5.70	5.55
14	4.88	3.67	10.00	20.75	3.58	0.14	4.47	4.46
15	4.54	3.30	8.80	20.32	3.96	0.18	4.10	5.56
16	4.30	3.28	8.11	20.23	3.55	0.10	4.20	4.27
17	2.43	2.78	6.94	19.65	3.10	0.24	2.95	2.76
18	4.96	3.38	10.71	20.35	2.89	0.08	4.30	2.49
19	5.45	3.50	10.74	20.83	3.92	0.08	4.09	4.74
20	4.72	3.73	9.53	20.50	3.61	0.24	4.60	—
21	6.80	4.00	15.45	20.55	4.60	0.30	6.00	5.30
22	4.43	3.15	9.93	21.60	4.40	0.15	4.76	5.54
Mean	4.78	3.32	10.54	20.51	3.60	0.18	4.44	4.50
2	3.38	2.56	6.90	16.88	2.93	19.93	2.39	2.78
3	2.74	2.59	3.09	12.99	2.73	37.44	1.85	2.34
4	6.12	5.22	12.54	28.35	5.58	44.70	3.82	5.26
5	11.96	4.55	11.27	23.97	10.48	41.97	6.30	4.47
6	3.28	2.82	7.08	14.25	2.93	44.92	2.57	2.46
7	2.60	2.44	3.54	11.60	3.03	44.70	1.74	2.00
8	3.09	2.64	5.00	14.24	3.07	45.75	2.26	2.60
9	2.88	2.73	4.08	13.19	2.64	43.17	2.14	2.47
10	2.80	2.68	2.95	14.40	2.80	39.15	2.40	2.63
11	2.81	2.68	3.88	13.75	2.70	36.78	2.08	2.44
12	3.30	2.71	5.85	14.45	2.94	44.63	2.82	2.98
13	2.90	2.60	5.20	14.20	2.60	14.80	2.15	2.40
14	3.00	2.68	5.30	13.70	2.78	38.70	2.27	2.60
15	3.04	2.76	2.72	13.16	3.12	34.50	2.16	2.30
16	6.66	3.11	8.15	17.07	3.89	50.15	4.57	3.31
17	9.91	5.00	18.60	17.60	5.10	39.00	4.78	3.10
18	2.68	2.55	4.14	13.24	2.20	40.32	1.80	4.26
19	3.15	2.58	3.84	13.50	2.72	44.27	1.98	2.45
20	3.06	2.77	5.29	13.87	3.27	43.83	2.49	—
21	5.85	4.85	7.95	17.40	5.30	43.20	4.20	5.10
22	2.73	2.50	3.20	11.90	2.75	40.28	2.00	2.48
Mean	4.19	3.10	6.22	15.41	3.60	39.62	2.80	3.02

2. The reports of all the collaborators confirmed the observations of Jacob, Rader and Tremearne (*2*) that the results obtained for citrate-insoluble P_2O_5 in basic slag and calcined phosphate vary greatly accord-

ing as filter paper is or is not present at the time of the citrate digestion. The mean values of the results submitted for all three samples analyzed (Table 7) were much lower when filter paper was present than when it was absent.

TABLE 7.—*Per cent citrate-insoluble P_2O_5 in standard phosphate samples as determined in the presence and absence of filter paper at the time of digestion*

COLLABORATOR	CALCINED PHOSPHATE, COOLED RAPIDLY, THROUGH 80 MESH		REVERTED CALCINED PHOSPHATE, THROUGH 80 MESH		BASIC SLAG, THROUGH 100 MESH	
	FILTER PAPER		FILTER PAPER		FILTER PAPER	
	PRESENT	ABSENT	PRESENT	ABSENT	PRESENT	ABSENT
1	3.65	9.93	20.85	25.53	3.45	6.30
2	3.10	13.25	20.55	26.65	3.05	7.40
3	3.17	7.85	20.12	23.93	3.43	6.00
4	3.20	13.05	20.37	26.05	3.45	7.40
5	3.27	10.20	20.13	24.78	4.02	7.06
6	2.93	4.78	19.73	22.58	3.26	4.82
8	3.20	7.77	20.37	23.64	3.53	6.00
9	2.95	7.08	19.74	26.93	3.40	5.38
10	3.13	5.65	20.38	22.50	3.88	5.30
11	3.04	11.23	20.87	25.26	3.57	6.67
12	3.32	10.03	21.21	25.27	3.39	5.93
13	4.10	11.60	21.80	25.65	4.00	7.90
14	3.67	6.41	20.75	24.87	3.58	6.21
15	3.30	7.58	20.32	21.74	3.96	4.97
16	3.28	5.51	20.23	22.32	3.55	5.21
17	2.78	7.38	19.65	23.27	3.10	5.68
18	3.38	7.90	20.35	24.63	2.89	5.57
19	3.50	8.80	20.83	23.28	3.92	5.42
20	3.73	5.75	20.50	23.08	3.61	6.10
21	4.00	11.40	20.55	25.50	4.60	7.00
22	3.15	12.53	21.60	22.66	4.40	5.83
Mean	3.33	8.84	20.52	24.29	3.62	6.10

3. The results obtained for citrate-insoluble P_2O_5 when Whatman filter No. 5 was used are in good agreement with those obtained with the Shimer filter (Table 8). This work, taken in conjunction with that reported last year (7), indicates that when provided with a suitable mat the Shimer filter is satisfactory for use in the filtration of citrate-insoluble residues.

4. The results submitted by most of the collaborators for the volumetric titration of phosphoric acid in a formaldehyde solution showed good agreement with those obtained by the official procedure (Table 9). In the cases of disagreement there is no way of knowing which procedure gave the more accurate results. Most of the collaborators expressed the

TABLE 8.—*Per cent citrate-insoluble P_2O_5 in standard phosphate samples as determined with different types of filters*

COLLABORATOR	CALCINED PHOSPHATE, COOLED RAPIDLY, THROUGH 80 MESH		REVERTED CALCINED PHOSPHATE, THROUGH 80 MESH		AMMONIATED SUPERPHOSPHATE	
	WHATMAN FILTER	SHIMER FILTER	WHATMAN FILTER	SHIMER FILTER	WHATMAN FILTER	SHIMER FILTER
1	3.65	3.65	20.85	20.80	5.05	4.95
2	3.10	3.78	20.55	20.88	4.35	4.73
3	3.17	3.29	20.12	20.08	4.10	4.03
4	3.20	3.40	20.37	20.55	3.80	4.20
5	3.27	3.08	20.13	19.93	4.75	3.98
6	2.93	2.95	19.73	19.71	3.57	3.59
8	3.20	3.42	20.37	20.70	5.51	5.27
9	2.95	2.91	19.74	19.48	4.10	3.50
10	3.13	3.27	20.38	20.63	4.55	5.10
11	3.04	3.11	20.87	19.58	3.90	4.85
12	3.32	3.70	21.21	20.82	4.88	4.96
13	4.10	3.60	21.80	20.35	5.55	6.10
14	3.67	3.52	20.75	20.30	4.47	4.51
15	3.30	3.32	20.32	20.31	5.56	5.58
16	3.28	3.18	20.23	20.37	4.27	4.20
17	2.78	2.84	19.65	20.05	2.76	2.90
18	3.38	3.48	20.35	20.03	—	—
19	3.50	3.66	20.83	21.70	4.74	5.10
21	4.00	3.60	20.55	20.60	5.30	4.60
22	3.15	3.39	21.60	20.75	5.54	5.90
Mean	3.31	3.36	20.52	20.38	4.57	4.63

opinion that the advantages claimed for the use of formaldehyde were not sufficiently marked to warrant its adoption.

COMPARISON OF RESULTS OBTAINED WITH CHEMICAL AND VEGETATIVE TESTS

The availabilities of the different phosphatic materials, as determined by the ammonium citrate and citric acid methods, are compared in Table 10 with their mean relative availabilities as shown by pot tests with acid soils. The data in this table indicate that the present ammonium citrate method is applicable to the evaluation of basic materials as well as of acidulated goods. The mean value found for the availability of the sample of basic slag by the citrate method is in exact agreement with that reported by the citric acid method, and there is little difference in the results obtained by either method for the other phosphates analyzed with the exception of calcium metaphosphate Sample 13. Judged by the vegetative tests, the citric acid method gives low results for the availability of this material.

The ammonium citrate method usually gives closely agreeing results

TABLE 9.—*Per cent citrate-insoluble P_2O_5 in standard phosphate samples as determined volumetrically with and without the presence of formaldehyde*

COLLABORATOR	CALCINED PHOSPHATE, COOLED RAPIDLY, 20–40 MESH		REVERTED CALCINED PHOSPHATE, THROUGH 80 MESH	
	PHENOLPHTHALEIN	PHENOLPHTHALEIN FORMALDEHYDE	PHENOLPHTHALEIN	PHENOLPHTHALEIN FORMALDEHYDE
1	14.55	15.20	20.85	20.51
2	10.15	13.15	20.55	23.43
3	6.90	6.83	20.12	20.04
4	10.90	10.96	20.37	20.43
5	10.02	11.04	20.13	19.80
6	8.33	8.27	19.73	19.70
8	9.04	9.03	20.37	20.33
9	10.88	10.34	19.74	19.23
10	9.55	9.23	20.38	20.00
11	10.66	10.48	20.87	20.58
12	13.50	13.38	21.21	21.03
13	17.75	17.80	21.80	21.75
14	10.00	10.01	20.75	20.58
15	8.80	8.75	20.32	20.36
16	8.11	8.10	20.23	20.08
17	6.94	6.85	19.65	19.67
18	10.32	10.26	19.96	19.72
19	10.74	11.27	20.83	21.68
21	15.45	15.44	20.55	20.54
22	9.93	9.94	21.60	21.59
Mean	10.62	10.81	20.50	20.55

TABLE 10.—*Comparison of availabilities of phosphatic materials as determined by chemical and vegetative tests*

SAMPLE NO.	PHOSPHATIC MATERIAL	RELATIVE AVAILABILITY BY POT TESTS[1]	AVAILABILITY BY—	
			CITRATE METHOD	CITRIC ACID METHOD
1	Monocalcium phosphate	100	100.0	100.0
5	Calcined phosphate, through 40 mesh	146	87.2	88.8
6	Calcined phosphate, through 80 mesh	134	91.1	91.7
8	Calcined phosphate, through 20-40 mesh	85	71.7	83.3
11	Calcined phosphate, reverted	111	44.6	58.4
12	Basic slag	110	80.5	80.5
13	Calcium metaphosphate	106	99.7	38.0
14	Fused phosphate rock	121	84.7	90.3
17	Ammoniated superphosphate	89[2]	76.1	84.0

[1] Average of tests on soils having pH values of 6 00 or below.
[2] Determined as sample A-8 in a previous publication (*8*)

in the hands of different analysts when it is used on superphosphate and mixtures containing superphosphate. The present investigation as well

as previous work (*8*) shows, however, that a much poorer agreement is often obtained when the method is applied in the analysis of certain other phosphatic materials. It would seem, therefore, that a further study should be made of the method if it is to find practical application in the evaluation of such materials.

SUMMARY

Finely ground calcined phosphate, calcium metaphosphate, and fused phosphate rock are as effective as monocalcium phosphate in promoting plant growth when applied to neutral or acid soils, but they are apparently less effective when applied to highly calcareous soils.

Calcined phosphate of 40 mesh or coarser is not so readily available to plants as the more finely divided material.

Calcined phosphate that has been reverted by slow cooling is more available to plants than is indicated by its solubility in either ammonium citrate or citric acid solution.

The present procedure for determining citrate-insoluble P_2O_5 may be applied in the evaluation of basic slag, calcined phosphate, fused phosphate rock, and calcium metaphosphate, but more difficulty is likely to be experienced in the application of the method to the analysis of these materials.

The availability of the P_2O_5 in calcined phosphate and fused phosphate rock as determined by the present official ammonium citrate method does not differ greatly from that found by the citric acid method.

The citric acid method gives low results for the availability of the P_2O_5 in calcium metaphosphate.

The values found for citrate-insoluble P_2O_5 in such basic materials as calcined phosphate and basic slag vary greatly according as filter paper is or is not present at the time of the citrate digestion.

The Shimer filter, when provided with a suitable mat, is a satisfactory filter for use in the filtration of citrate-insoluble residues.

RECOMMENDATIONS

It is recommended[1] that a further study be made of the nature of the citrate-insoluble components of phosphate materials with a view to improving the method of determining the availability of such materials.

LITERATURE CITED

(*1*) JACOB, BARTHOLOMEW, BROWN, PIERRE, REID, and TIDMORE, *J. Agr. Research*, 50, 837–48 (1935).

(*2*) JACOB, RADER, and TREMEARNE, *J. Assoc. Official Agr. Chemists*, 19, 449–72 (1936).

(*3*) MARSHALL, REYNOLDS, JACOB, and RADER, *Ind. Eng. Chem.*, 27, 205–9 (1935).

(*4*) MARSHALL, REYNOLDS, JACOBS, and TREMEARNE, *Ibid.*, 29, 1937. To be published.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 50 (1937).

(5) Reynolds, Jacob, Marshall, and Rader, *Ibid.*, **27**, 87–91 (1935); **28**, 678–82 (1936).
(6) Reynolds, Jacob, and Rader, *Ibid.*, **26**, 406–12 (1934).
(7) Ross and Hardesty, *J. Assoc. Official Agr. Chemists*, **19**, 269–79 (1936).
(8) Ross, Jacob, and Beeson, *Ibid.*, **15**, 227–66 (1932).
(9) *J. Assoc. Official Agr. Chemists*, **15**, 44 (1932).

REPORT ON NITROGEN*

By A. L. Prince (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

There were three recommendations approved last year by this Association as official (first action). The first of these concerned the determination of water-insoluble nitrogen in cyanamid. A method was proposed and considerable collaborative work was done. No further collaborative work was carried on this year. The other two recommendations were mainly of an editorial nature, concerning the use of alcohol to moisten fertilizer mixtures greasy in character prior to washing with water for the determination of water-insoluble nitrogen.

It has been suggested by several chemists connected with the fertilizer trade that more specific directions should be given in the methods for the determination of insoluble nitrogen both in raw materials and in mixed fertilizers. The main question raised related to the type of filter paper to be used, and the volume to which the fertilizer material should be leached. In the present methods no particular type of paper has been specified. Also under paragraph **34 (b),** 1935 edition of *Methods of Analysis,* the volume of washing is confined to 200 cc., while under the Nitrogen Activity Methods, paragraph **37,** 250 cc. is given as the amount of water to which the sample is leached. In the latter case, however, only a preliminary test is made to determine the amount of sample that would be necessary in order to yield 50 mg. of water-insoluble organic nitrogen. Under paragraph **34(b)** the weight of sample taken and the filtrate volume (2 grams leached to 200 cc.) has been chosen with a view to obtaining convenient aliquot parts for subsequent determinations.

Consequently the associate referee made some analyses this year on a sample of tankage and on a mixed fertilizer for water-insoluble nitrogen, using three common types of filter paper and three different sample weights. Also the washing was carried on to a volume of 200 and 250 cc., respectively. The sample of tankage used had a total nitrogen content of 7.30 per cent and the mixed fertilizer of 6.16 per cent. The three types of filter paper used were Whatman No. 1, Whatman No. 2, and C. S. and

* Journal series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

TABLE 1.—*Water-insoluble nitrogen in a sample of tankage and mixed fertilizers*

WEIGHT OF SAMPLE USED	TYPE OF FILTER PAPER	VOLUME LEACHED TO	AVERAGE WATER—INSOLUBLE NITROGEN*	
			TANKAGE	MIXED FERTILIZER
grams		*cc.*	*per cent*	*per cent*
1.0	Whatman No. 1	200	5.23	0.36
1.0	Whatman No. 2	200	5.25	0.36
1.0	C.S. and S. No. 597	200	5.45	0.36
1.0	Whatman No. 1	250	5.18	0.32
1.0	Whatman No. 2	250	5.34	0.36
1.0	C.S. and S. No. 597	250	5.31	0.37
General average of 1.0 gram samples			5.29	0.36
2.0	Whatman No. 1	200	5.25	0.35
2.0	Whatman No. 2	200	5.23	0.35
2.0	C.S. and S. No. 597	200	5.32	0.38
2.0	Whatman No. 1	250	5.20	0.31
2.0	Whatman No. 2	250	5.23	0.34
2.0	C.S. and S. No. 597	250	5.37	0.35
General average of 2.0 gram samples			5.27	0.35
2.5	Whatman No. 1	200	5.31	0.39
2.5	Whatman No. 2	200	5.37	0.43
2.5	C.S. and S. No. 597	200	5.24	0.49
2.5	Whatman No. 1	250	5.29	0.32
2.5	Whatman No. 2	250	5.34	0.33
2.5	C.S. and S. No. 597	250	5.34	0.33
General average of 2.5 gram samples			5.32	0.37

* Average of duplicates.

S. No. 597. Other grades of qualitative paper might have been tried but it was thought best to confine the comparative tests to those grades of filter paper in most common use in all laboratories. Quantitative paper was ruled out as being too expensive for the purpose. The weights of the samples taken for analysis were 1, 2, and 2.5 grams. Each sample was moistened on the filter paper with 5–7 cc. of ethyl alcohol prior to washing with water. The average of duplicate determinations may be seen in Table 1, as well as the general average when different initial sample weights were used.

It is quite apparent that the differences in results obtained are slight in all cases, and furthermore that these differences are irregular. However, there appeared to be a general tendency for the C. S. and S. No. 597 filter paper to yield slightly higher results for water-insoluble nitrogen, and the fact that this paper filtered most rapidly in all cases may account for the differences (Table 2). Thus, the average per cent water-insoluble nitrogen in tankage with Whatman No. 1 paper leached to 200 cc. was

TABLE 2.—*Results showing effect of using different filter papers**

TYPE OF FILTER PAPER	WATER-INSOLUBLE NITROGEN			
	TANKAGE		MIXED FERTILIZER	
	LEACHED TO 200 cc.	LEACHED TO 250 cc.	LEACHED TO 200 cc.	LEACHED TO 250 cc.
	per cent	*per cent*	*per cent*	*per cent*
Whatman No. 1	5.26	5.22	0.36	0.32
Whatman No. 2	5.28	5.30	0.38	0.34
C. S. and S. No. 597	5.34	5.34	0.41	0.35

* Average obtained with 1, 2, and 2.5 grams of fertilizer.

5.26; for Whatman No. 2, 5.28; and for C. S. and S. No. 597, 5.34. With the sample of mixed fertilizer the results were 0.36, 0.38, and 0.41 per cent water-insoluble nitrogen, respectively. The same tendency may be noted in Columns 2 and 4 of Table 2, where the leaching was continued to 250 cc.

In Column 4, there is also an indication of slightly less water-insoluble nitrogen in the mixed fertilizer with increased washing. Similar results may be noted in the second and last column of Table 3.

TABLE 3.—*Results showing effect of varying size of sample**

SAMPLE	WATER-INSOLUBLE NITROGEN			
	TANKAGE		MIXED FERTILIZER	
	LEACHED TO 200 cc.	LEACHED TO 250 cc.	LEACHED TO 200 cc.	LEACHED TO 250 cc.
grams	*per cent*	*per cent*	*per cent*	*per cent*
1.0	5.31	5.28	0.36	0.35
2.0	5.27	5.27	0.36	0.33
2.5	5.31	5.32	0.40	0.33

* Average obtained by using Whatman No 1, Whatman No 2, and C. S. and S. No 597 filter paper.

Although the data here presented are rather meager and from only two fertilizer materials, the slight differences that occurred in the comparison of three common grades of filter paper explain why no specific mention has been made of this point in the past and present methods. However, for the sake of uniformity among the chemists in carrying out this procedure, it might be well to specify a particular grade of filter paper. Hence, it is suggested that Whatman No. 2 filter paper be used for this purpose. The rapidity of the C. S. and S. No. 597 paper is a desirable feature but the results show that Whatman No. 2 allowed more complete removal of the water-soluble nitrogen. Whatman No. 1 paper proved to be the slowest of the three filter papers tried. It would also appear from the data that a 2 gram sample leached to 200 cc. was sufficient and a convenient quantity to work with.

RECOMMENDATIONS[1]

It is recommended—

(1) That the first part of the first sentence under **34 (b)**, p. 27 of the 1935 edition of *Methods of Analysis* be changed to read: "Weigh out 2.0 g. of the fertilizer mixture on a 11 cm. Whatman No. 2 filter paper, etc.".

(2) That the method for the determination of water-insoluble nitrogen in cyanamid be further studied collaboratively with a view to its final adoption as official.

REPORT ON MAGNESIUM IN FERTILIZERS*

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*†

The work of the year has been a continuation of the lines of study reported in 1935, *This Journal*, **19**, 288 (1936). The progress made and the status of each phase are discussed under the appropriate headings.

TOTAL MAGNESIUM OXIDE

This method is intended to measure all the magnesium in a fertilizer mixture that could conceivably be useful to plants, without regard to the rate at which it becomes available. The term "total" is applicable only in this respect, for the hydrochloric-nitric acid mixture used may not dissolve silicates of magnesia that may be present. Possibly "Acid-soluble magnesium oxide" is a better term.

The method published by Hoffman[2] for the determination of magnesia in phosphate rock and recommended last year for use with mixed fertilizers was studied collaboratively. Preliminary work shows that the size of aliquot part must be restricted to contain not more than 0.03 gram of magnesium oxide, and with this single change the directions sent to collaborators were identical with those published in the previous report and are not repeated here. Briefly, the method includes dissolving the sample by boiling in 10 cc. of hydrochloric acid plus 30 cc. of nitric acid, dehydration and separation of silicon dioxide, precipitation and removal of calcium sulfate in alcohol, and a double precipitation of magnesium ammonium phosphate in the alcohol-free filtrate. Iron and aluminum are held in solution as citrates during the first magnesium ammonium phosphate precipitation. If manganese is present, it is determined in the ignited precipitate and subtracted as manganese pyrophosphate.

The four samples, taken from commercial stocks to represent actual

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 50 (1937).

* Contribution No. 499 of the Station.

† The Associate Referee is indebted to the collaborators listed in another section of this report; to representatives of several commercial firms, who have furnished samples of their products; and especially to Mr. E. J. Deszyck of this laboratory for analytical assistance and aid in planning the work, and for interpretation of the results.

[2] *Bur. Standards J. Research*, **9**, 487–491, (1932).

curing conditions, contain most of the common fertilizer ingredients and magnesium carriers, with only traces of manganese. The large samples were ground to pass a 0.5 mm. sieve and were thoroughly mixed before subdivision. The results (Table 1) show satisfactory agreement in most instances, although more experience with the procedure would doubtless produce greater uniformity. Four of the 11 analysts report results consistently above the average for each sample and three fall consistently below. The maximum deviations are rather large, especially for Sample L. It must be agreed that the technic is not entirely satisfactory, for after two years of almost contant use of the method in this laboratory unexplainable discrepancies appear all too frequently. With reasonable care in verification of results by repetition, the analyst should obtain as much accuracy as with other procedures in fertilizer analysis.

TABLE 1.—*Collaborators' results for acid-soluble MgO in mixed fertilizers*

COLLABORATORS	SAMPLE B 6-8-6	SAMPLE G 4-12-4	SAMPLE K 12-16-12	SAMPLE L 8-12-20
	per cent	*per cent*	*per cent*	*per cent*
	Hoffman method as recommended			
J. Rich Holland, Wiley & Co., Inc.	4.93	3.12	2.28	3.35
W. Catesby Jones, Va. Dept. of Agr.	4.87	3.16	2.31	3.82
O. I. Struve, Eastern States Co-op. Milling Corp.	4.80	2.86	1.92	3.15
R. C. Koch, Swift & Co.	4.56	3.10	2.12	3.35
C. A. Butt and A. O. Hallman, International Agr. Corp.	4.71	2.82	1.95	3.06
J. W. Kuzmeski, Mass. Agr. Exp. Sta.	4.80	3.02	2.03	3.36
C. Harry White, Maine Agr. Exp. Sta.	4.75	2.87	2.09	3.25
Millard G. Moore, Maine Agr. Exp. Sta.	4.80	2.90	2.00	3.15
J. G. Thompson, Lawrence Portland Cement Co.	4.76	2.95	2.06	3.40
W. A. Ryder, F. S. Royster Guano Co.	5.00	3.18	2.28	3.46
E. J. Deszyck, R. I. Agr. Exp. Sta.	4.90	2.90	2.09	3.50
Average	4.81	2.99	2.10	3.36
Average deviation	0.07	0.12	0.12	0.15
	Bartlett-Tobey method			
C. Harry White	4.91	3.18	2.01	3.42
Millard G. Moore	4.84	3.00	2.05	3.30
J. W. Kuzmeski	5.05	3.24	2.24	3.49
E. J. Deszyck	4.53	3.05	1.99	3.38
Average	4.83	3.12	2.07	3.40

In addition to the results by the Hoffman method, four of the analysts presented results by an unpublished method for acid-soluble magnesium oxide suggested by Bartlett and Tobey, and used by them at the Maine

Agricultural Experiment Station. This method represents an attempt to shorten the customary separation of calcium and magnesium as oxalates. The fertilizer is extracted with 1 per cent hydrochloric acid, calcium oxalate is precipitated from an aliquot at *p*H 5, and magnesium ammonium phosphate is precipitated from the filtrate in the presence of citric acid. Commonly a single precipitation of magnesium is used, but reprecipitation is suggested at the discretion of the analyst. The weight of the ignited precipitate is corrected for the manganese pyrophosphate as in the Hoffman method. This is a much more rapid procedure than that of Hoffman, but purposely eliminates the complete destruction of organic matter, separation of silica, double precipitation of calcium oxalate, and in most instances the second precipitation of magnesium, precautions provided by standard procedures. The time of standing after each precipitation is shorter than that usually recommended.

This method was tried last year by a single analyst, who obtained satisfactory results with four samples and low results with three others. This year four analysts present excellent agreement for each of the four samples distributed (Table 1). The method would save much time if it can be shown to be sufficiently accurate, and should be studied intensively next year.

ACTIVE MAGNESIUM

The most important method is one for determining the magnesium available to plants during a single cropping season, but this method cannot be written until the relative crop response to different magnesium compounds is known. The evidence thus far is insufficient, even for the ingredients most commonly used. Progress has been made during the past year, for field trials are under way in many places and with different crops.[1] Pot experiments are being organized, notably a comprehensive test with several soils at the Indiana Agricultural Experiment Station. Magnesium presents all the difficulties that have been experienced with other elements, and single values for "availability" applicable to all conditions cannot be expected. Average values from crop response in several soils must be accepted as soon as possible as a basis for the laboratory method, and then the usual process of modification to meet new evidence must follow.

The laboratory portion of the work, selection of a solvent that will remove fractions of the magnesium from different ingredients in proportion to plant response to these ingredients, will not be simple, even after the proportions are known. In order to have a basis from which to start, a study of the action of a number of solvents with several magnesium compounds was reported last year, and this work was continued. From previous evidence, citric acid and ammonium citrate solutions seemed promising, for the more soluble compounds could be recovered completely,

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[1] Va. Truck Exp. Sta. Bull. 89, (1935).

and the fractions dissolved from less soluble materials such as dolomite could be varied by changing such conditions as time, temperature, volume, and acidity.

Ammonium oxalate solutions appeared to have the same qualifications as ammonium citrate and the added advantage of separating the calcium during the magnesium extraction. Subsequent work with dolomite alone has shown that the precipitated calcium oxalate tends to coat the particles, stopping solvent action before sufficient magnesium has dissolved, thus limiting the adaptability of the process. Possibly the other ingredients in a mixture might serve as absorbents for the calcium oxalate, for the precipitate tends to cling to the rough sides of etched beakers, and greater solvency was noted under this condition. However, the study of this solvent has been dropped for the time, to allow for work with ammonium citrate—citric acid mixtures.

After considerable preliminary study a solution containing 4 grams of citric acid in 100 cc. and adjusted to *p*H 4 with concentrated ammonium hydroxide was chosen as the most promising. It is strongly buffered against *p*H change during the extraction, easily prepared by using colorimetric or potentiometric methods for *p*H, and its solvent action can be varied over a wide range by changing the *p*H. The specific gravity of the solution used was 1.0188 at 20° C.

TABLE 2.—*Solubility of various magnesic limestones in 4 per cent citric acid* adjusted to pH 4 with NH_4OH*

SAMPLE NUMBER	ACID-SOLUBLE MgO	PERCENTAGES OF THE ACID-SOLUBLE MgO RECOVERED 80–100-MESH SEPARATES				
		60 MIN.	75 MIN.	90 MIN.	105 MIN.	120 MIN.
	per cent					
15B	19.66	37	53	60	64	73
5721	21.45	37	49	60	—	77
5834	21.47	39	48	56	—	73
9	17.27	46	47	55	60	68
3	22.08	49	49	59	66	81
21	22.46	53	53	52	65	76
6	12.18	54	58	67	65	83
5764	22.02	56	64	73	—	93
5818	21.98	58	64	73	—	96
22	12.48	59	65	75	73	91
18	22.26	61	66	81	79	89
23	21.50	89	—	95	98	—
12A	18.88	97	99	100	100	100
		60–100-mesh separates				
24	20.09	44	47	52	58	74
3808	20.57	70	78	81	86	100
25	17.22	72	78	85	90	104

* 0.2 g. samples in 100 cc. of solution maintained at 90–95° C.

The rate of solubility of different magnesic limestones in this solution was studied by treating 0.2 gram samples in 100 cc. at 90–95° C. for different time periods. Sixteen different products were collected from various points from Alabama to Maine. To control the effect of particle size, most of the comparisons are for 80 to 100-mesh separates. The data in Table 2 show wide differences in the rates of solubility of these materials, confirming the results obtained by Siems[1] with 0.1 N hydrochloric acid, but indicate greater differentiation with this weak acid solvent. It remains to be shown that the differences found in solubility rates are reflected in the reactions of these materials in the fertilizer curing pile, in the soil, and in plant response. The results show that the differences are decreased by lengthening the periods of treatment, making it possible to choose various relationships to correspond with the qualities to be found in future work.

The action of this solution was tested further by studying two fertilizer mixtures made in this laboratory. The mixtures were intended to represent general practice, and the object was to study the effect of the components on water-soluble magnesium oxide, as well as on solubility in the citric acid solution.

The base of the first mixture contained common ingredients, all ground to pass a 1 mm. sieve before being mixed (see end of Table 3).

A considerable quantity of this base was prepared and mixed thoroughly; 22 equal portions were taken, and to each portion was added a quantity of some magnesium carrier to supply 3 per cent of magnesium oxide in the final mixture. Sand was added when necessary, to make 1500 grams of a 5–8–7 fertilizer carrying 3 per cent of magnesium oxide equivalent. Each portion was mixed thoroughly and spread out on oilcloth, and a measured quantity of water was sprinkled over the pile to make 5 per cent of moisture. To simulate as best possible the conditions of the curing pile, the mixtures were placed in glass jars and kept at 60° C. in a large constant-temperature oven, as advised by W. H. Ross.[2] No attempt was made to develop or control pressure. Moisture was determined by distillation from xylene, and the weight of jar and sample with 5 per cent of moisture was calculated. It was found that a pan of water placed on the electric hot-plate that heats the oven maintained sufficient humidity to prevent material changes in the moisture content of the samples. At the end of the curing period, the samples were allowed to come to equilibrium with the air at room temperature and were reground to pass a 1 mm. sieve.

Acid-soluble magnesium oxide was determined by the Hoffman method; the citrate-soluble fraction by a modification of this method, the extracting solution described previously being used; and water-soluble magnesium oxide by leaching with water and by boiling for varying periods of time. The magnesium oxide found in the base mixture alone was

[1] Paper read before Am. Soc. Agron., Dec., 1935.
[2] Private communication.

TABLE 3.—*Recovery of MgO from various carriers added to a 5–8–7 fertilizer*[1] *by different solvents*

MAGNESIUM CARRIER	RECOVERY OF ADDED MgO (PER CENT)						
	STRONG ACIDS[2]	CITRIC ACID NH_4 CITRATE[3]	WATER[4]				
			A	B	C	D	E
Calcined kieserite	97	93	84	84	84	86	87
Sulfate of potash magnesia	99	95	86	87	87	87	87
$MgNH_4PO_4$	87	94	42	58	77	72	73
Kieserite ½, sulfate of potash magnesia ½	97	98	89	89	94	94	90
Kieserite ½, $MgNH_4PO_4$ ½	88	88	69	73	81	73	77
Sulfate of potash magnesia ½, $MgNH_4PO_4$ ½	97	92	70	71	82	81	81
Magnesic limestones							
12A, 100–120-mesh	98	83	3	4	—	—	—
12A, 80–100-mesh	101	71	3	4	—	—	—
12A, 60– 80-mesh	101	77	3	3	—	—	—
12A, 20– 60-mesh	102	59	4	4	—	—	—
15B, 100–120-mesh	101	23	4	4	—	—	—
15B, 80–100-mesh	97	19	4	4	—	—	—
15B, 60– 80-mesh	100	18	4	3	—	—	—
15B, 20– 60-mesh	100	16	4	2	—	—	—
9, 80–100-mesh	102	31	5	5	—	—	—
21, 80–100-mesh	99	24	4	2	—	—	—
3, 80–100-mesh	103	20	4	3	—	—	—
12A, 80–100-mesh ½, kieserite ½	96	85	41	41	48	45	41
12A, 80-100-mesh ½, sulfate of potash magnesia ½	96	86	45	45	50	47	48
12A, 80–100 mesh ½, $MgNH_4PO_4$ ½	97	73	31	36	43	41	38
Serpentine	108	75	21	22	—	—	—
Olivine	102	30	13	12	—	—	—

[1] The carriers supplied 3% MgO in the final mixture. 1500 g. of each mixture was cured for 30 days at 60°C. in a humid oven.
[2] 0.5 g. with 10 cc. of HCl plus 30 cc of HNO_3.
[3] 1 g. with 100 cc. of 4% citric acid adjusted to pH 4 with NH_4OH, 90 min, 90–95°C, shaking every 5 min.
[4] A—Leached 1 g. with 200 cc. of boiling water.
B—Boiled 1 g. with 150 cc. of water for 30 min.
C—Boiled 1 g. with 200 cc. of water for 60 min.
D—Boiled 1 g. with 250 cc. of water for 60 min.
E—Boiled 1 g. with 200 cc of water for 90 min.

Base Mixture:	*lbs.*	*Base Mixture:*	*lbs.*
Sulfate of ammonia	250	Diammonium phosphate	120
Nitrate of soda	100	Superphosphate	600
Cottonseed meal	126	Muriate of potash	280

subtracted from that found after special magnesium carriers were added and the percentages recovered were calculated. The treatments and results are reported in Table 3.

Within the limits of error of the method, the magnesium oxide was recovered completely by strong acids except for the treatment containing magnesium ammonium phosphate, either alone or with calcined kieserite. The cause of this discrepancy was not discovered. The citric acid-ammonium citrate solution recovered substantially all the water-soluble

forms of magnesia. All the acid-soluble magnesia when magnesium ammonium phosphate was added was also soluble in this solution. The relative solubility of the magnesic limestones alone in the citrate solution (Table 2) was reflected by the recovery from the mixtures, but the quantities were much less than was expected. If this depressed solubility indicates decreased availability to plants, and varies with different mixtures, the difficulty in devising a satisfactory method for availability will be increased, for it will be almost impossible to predict the effect of dolomite except in the final mixtures by any simple uniform laboratory procedure. The results show decreased recovery from coarser limestone particles. The recoveries of magnesia from additions of equal quantities of magnesium added in magnesic limestone and calcined kieserite or sulfate of potash magnesia were approximately the same as those from these ingredients used separately. Magnesia in the sample of serpentine used was as soluble as that in any of the limestones, and the olivine was much like the more insoluble forms, but these samples are insufficient to justify conclusions.

Boiling 1 gram samples with water for 60 minutes was the most effective of the water treatments tried, but it recovered less than 90 per cent of the water-soluble magnesia added. This fact has been noted before, although approximately all the water-soluble forms have been recovered from certain commercial mixtures. In this instance, the diammonium phosphate used may have caused the formation of insoluble phosphates of magnesium. The decreased solubility may be more general than is usually recognized, for many fertilizer ingredients carry small quantities of soluble magnesium, and these aid in making up the recoveries calculated from the additions of water-soluble carriers alone. No appreciable quantities of the magnesium limestones in these mixtures have reacted with the ammonium compounds in the base to create water-soluble magnesium.

A second mixture was prepared to represent a fertilizer base. The ingredients and proportions are given under Table 4.

Ammonia liquor in the small quantities needed could not be secured for no small pressure containers were available. By advice of F. W. Parker, E. I. DuPont de Nemours & Co., Inc., a mixture of ingredients in the following proportions was used to represent the commercial product:

	grams
Urea	20
Conc. ammonium hydroxide	69
Ammonium carbonate	11

This liquor contains 26 per cent nitrogen, less than the most dilute commercial product, but it is as concentrated as could be prepared with ammonium hydroxide at one atmosphere.

TABLE 4.—*Recovery of MgO from various carriers added to a 4-13.5-0 base,*[1] *by different solvents*

MAGNESIUM CARRIER	RECOVERY OF MgO ADDED (PER CENT)						CITRATE-INSOLUBLE P_2O_5
	CITRIC ACID NH_4-CITRATE[2]		WATER[3]				
	FROM MIXTURE	FROM LIMESTONE RESIDUE[4]	A	B	C	D	
							per cent
Calcined kieserite	98	—	75	85	85	83	—
Magnesic limestones							
12A, 100–120-mesh	84	74	17	38	35	34	4.33
12A, 80–100-mesh	86	81	14	26	25	24	2.97
12A, 60– 80-mesh	81	75	16	25	27	30	—
12A, 20– 60-mesh	87	80	23	34	36	39	—
15B, 100–120-mesh	60	42	25	31	27	28	3.43
15B, 80–100-mesh	35	23	3	16	12	13	2.81
15B, 60– 80-mesh	54	34	16	30	22	24	—
15B, 20– 60-mesh	32	22	3	13	9	11	—
3, 80–100-mesh	43	27	13	22	16	18	2.98
9, 80–100-mesh	45	27	15	25	23	22	2.84
21, 80–100-mesh	47	34	12	19	18	18	3.06
22, 80 100-mesh	40	—	—	—	—	—	—
Olivine	52	26	29	35	38	38	—
Base Mixture	—	—	—	—	—	—	2.74

[1] The carriers supplied 1.3% MgO in the final mixture. 800 grams of each mixture was cured for 30 days at 60°C. in a humid oven.
[2] 1 g with 4% citric acid adjusted to pH 4 with NH_4OH, 90 min, 90–95°C, shaking every 5 minutes.
[3] A—Leached 1 g. with 200 cc of boiling water.
B—Boiled 1 g. with 200 cc. of water for 60 min.
C—Like B, but 90 min.
D—Like B, but 120 min.
[4] Calculated on the basis of the limestone unreacted during the curing process; Column "B" used as a measure of the reacted fraction.

Base Mixture:	*lbs.*
Superphosphate (18% P_2O_5).	1500
Urea-ammonia liquor (25.7% N).	242
Ammonium sulfate.	98

Magnesium compounds were added to portions of this base to supply 1.3 per cent magnesium oxide in the final mixture, and the various treatments, 750 grams in this instance, were "cured" as described for the 5–8–7 mixture discussed previously. Because of an error in directions the mixtures, rather moist from the ammonia liquor, were not dried to 5 per cent moisture, but contained approximately 10 per cent throughout the 30 days in the oven. Thus, the reactions may have gone farther than would have happened at a lower moisture content.

Magnesium soluble in the citric acid-ammonium citrate solution, and water-soluble magnesia were determined (Table 4). As was expected, a portion of the magnesium in the limestones was made water-soluble during the curing process. These results should not be translated quantitatively to commercial practice, for the mixtures were probably more moist than would happen in a curing pile, and the other conditions would not be duplicated exactly. In principle, however, it is probably true that the

various commercial ammonia liquors react in some degree with magnesic limestones. In this instance the amount of water-soluble magnesium would be very small after the additions of other ingredients to make the base a complete fertilizer of the usual commercial grades, probably less than 0.3 per cent. Leaching with water gave lower results than boiling in every instance. This appears to be incomplete solution rather than reaction between ammonium salts and the limestone during boiling, for the recoveries were not increased by 90 and 120 minute boiling periods as compared with 60 minutes. The limestone that was most soluble in the acid solution (Table 2) reacted with the ammonium compounds to a slightly greater extent than did the others, but particle size had no consistent effect. Further evidence of reaction during the oven treatment of these products is shown by small but significant increases in the citrate-insoluble phosphoric acid (Table 4). These increases, like those in water-soluble magnesia, are probably greater than would occur under commercial conditions, but the two types of reaction seem to be related.

The citrate solubility of the magnesium compounds in these mixtures was high, but must be ascribed in part to the reactions during "curing." The unreacted portions of the limestones were calculated by subtracting the water-soluble magnesia from the total. If it is assumed that the difference between the amounts of citrate-soluble and water-soluble magnesia is the magnesia dissolved from limestone that was not decomposed in "curing," the citrate solubility of this limestone residue could be calculated. On this basis, the recovery of the magnesium in the limestones was much the same as that found in the 5–8–7 mixture, and again less than was expected from results with the limestone alone. The differences among different limestones were rather similar to those noted previously, but particle size had less effect.

These results help to outline the problem and supply a group of mixtures for use with pot cultures. They will be significant when correlations with crop response are available, and will help to show in what way the procedure should be changed to give the desired relationships.

WATER-SOLUBLE MAGNESIA

This fraction is of considerable agronomic and commercial interest, for it represents a portion that all agree is available, at least before reaction with the soil colloidal complex. No official method should be proposed for this determination until a procedure is accepted for "available" magnesia that does justice to all common magnesium carriers, but considerable data have been obtained in anticipation of later needs.

The data in Table 5 show that in many instances leaching commercial fertilizer mixtures with hot water dissolved less magnesia than did boiling with water. This was also true for one of the laboratory mixtures (Table 4). There seems to be a decrease during storage in the magnesia found by leaching. This was recoverable in part by boiling with water.

TABLE 5.—*Water-soluble MgO obtained by leaching commercial fertilizers with hot water compared with boiling in water*

SAMPLE	MgO BY LEACHING 1 G. WITH 250 CC. OF BOILING WATER (PER CENT)						MgO BY BOILING 1 G. IN 150 CC. OF WATER FOR 30 MIN.
	JULY 20	AUG. 4	AUG. 10	AUG. 17	NOV. 1	APRIL 2	APRIL 2
							per cent
4–8–10	2.07	1.98				1.10	2.05
7–16–20	1.70	1.60	1.46		1.29	1.16	1.44
4–12–4	1.38					0.91	1.58
8–16–14	1.59	1.50				0.97	1.36
8–16–20	1.60	1.43	1.35	1.51	1.33	1.26	1.38
8–12–20	1.88	1.38	1.28	1.12	1.23	1.37	1.62
8–12–20						0.68	0.84
6–12–12						0.94	0.97

Of the magnesia in water-soluble compounds added to the two laboratory mixtures, only about 85 per cent could be recovered by any of the water treatments tried (Tables 3, 4). Unless it can be shown that the remainder is less available to plants than the portion that remains soluble in water, some other solvent should be chosen, as has been done for "water-soluble" potash.

TABLE 6.—*Composition of sulfate of potash magnesia (per cent)*

SAMPLE	MOISTURE	MgO	K_2O	SO_4	CaO	SiO_2	Fe_2O_3 PLUS Al_2O_3	CO_2
1	4.02	9.83	25.39	57.57	6.60	0.29	0.38	0.012
2	3.63	9.23	28.66	55.69	5.22	0.34	0.31	0.022
3	3.61	10.36	26.05	56.05	5.90	0.32	0.33	0.035

MAGNESIA IN WATER-SOLUBLE INGREDIENTS OF FERTILIZERS

Sulfate of potash magnesia is a common source of magnesia for mixed fertilizers. While it has been generally assumed that this product is soluble in water, most laboratories have reported only 75–80 per cent of the magnesia present. The composition of the material as determined in this laboratory (Table 6) does not indicate important quantities of water-insoluble magnesia. Three samples of the product, selected at random, were leached with boiling water and also placed directly in water at different temperatures and volumes, and for varying lengths of time. The results (Table 7) show that leaching with hot water does not recover all the acid-soluble magnesia. Temperature seems to be the most important factor, and substantially all the magnesia was made soluble by boiling a 1 gram sample in 100 cc. of water for 30 minutes. The apparent failure to recover 100 per cent of the magnesia in Sample 3 may be accounted for by analytical errors.

The commercial carriers of magnesium sulfate are made soluble by leaching, but boiling is the easier process. The procedure published below

TABLE 7.—*Effect of time, volume, and temperature on the solubility of sulfate of potash magnesia in water*
(Results for water solubility expressed as per cent recovery of the acid-soluble MgO present.)

SAMPLE NO.	ACID-SOLUBLE MgO	LEACHED 1 G. WITH 200 CC. OF BOILING WATER	1 G. SAMPLE PLACED IN WATER									
			TEMPERATURE	50 CC. WATER			100 CC. WATER			200 CC. WATER		
				30 MIN.	60 MIN.	120 MIN.	30 MIN.	60 MIN	120 MIN.	30 MIN.	60 MIN.	120 MIN.
1	9.83	97	25°C.	89	91	89	90	91	90	89	93	94
			65°C.	98	97	96	101	99	98	98	101	97
			Boiling	101	99	101	100	102	100	99	99	101
2	9.23	89	25°C.	73	80	82	77	80	79	76	82	89
			65°C.	84	89	93	89	92	95	90	96	98
			Boiling	98	100	99	99	102	100	99	98	100
3	10.36	89	25°C.	77	81	83	79	82	82	82	84	90
			65°C.	87	86	95	87	90	99	89	92	98
			Boiling	94	96	95	96	97	99	96	97	98

NOTE: Samples at 25°C. and 65°C. were shaken at 5 min. intervals.

is based on these data, and it is advocated as a method that will give more uniform results from different laboratories, and results that do justice to the products mentioned. It is not intended for use with fertilizer mixtures, but only with the water-soluble ingredients discussed in this section. This method will be studied collaboratively next year.

MAGNESIA IN WATER-SOLUBLE COMPOUNDS

(Applicable to sulfate of potash-magnesia, sulfate of magnesia, and kieserite)

Weigh a 1 gram sample into a 250 cc. volumetric flask. Add 175 cc. of water, and boil for 30 minutes; cool, and dilute to volume with water. Pipet an aliquot containing approximately 30 mg. of MgO into a beaker and proceed as directed in XII, 10, for separation of calcium, and 12, p. 124, for precipitation of the magnesium, *Methods of Analysis, A.O.A.C.*, 1935.

Or evaporate the aliquot to a volume of 10 cc. add 6 cc. of H_2SO_4 (1+1) and 100 cc. of 95% alcohol, and proceed as directed in the method for total magnesia, II, 54, *Methods of Analysis, A.O.A.C.*, 1935, beginning with "stir so that the $CaSO_4$ is well dispersed throughout the liquid." Calculate as percentage of MgO.

MANGANESE

All resources available during the year were expended on the magnesium problem. Next year a part of the work will be directed toward manganese in the hope of outlining the problem and devising a method for the acid-soluble portion.

RECOMMENDATIONS[1]

It is recommended—

(1) That the title of the method published last year, *Methods of Analysis, A.O.A.C.*, 1935, 54, be changed from "Total Magnesia" to "Acid-Soluble Magnesia," and that the first two sentences be changed to read: "Weigh 2 g. of sample into a 200 cc. volumetric flask, add 10 cc. of HCl and 30 cc. of HNO_3, boil gently for 30 min., cool, and dilute to volume with H_2O. Pipet an aliquot containing not more than 30 mg. of MgO into a 250 cc. beaker, add 6 cc. of H_2SO_4 (1+1), evaporate until white fumes appear," and that with these minor changes, the method be adopted as official (first action).

(2) That the method presented for the determination of magnesia in water-soluble compounds, used as sources of magnesium in mixed fertilizers, be studied collaboratively.

(3) That the study of methods for active magnesia in mixed fertilizers be continued, with emphasis on the search for shorter procedures for determining magnesia after it is dissolved from fertilizers.

(4) That a method for acid-soluble manganese be devised.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 51, 67 (1937).

REPORT ON POTASH

By H. R. KRAYBILL (Purdue University Agricultural Experiment Station, West LaFayette, Ind.), *Associate Referee*

No collaborative studies were made on the method for the determination of potash in commercial fertilizers during the last year. The Associate Referee corresponded with a number of laboratories in which the modified method adopted last year has been used. A number of workers have reported that when the samples are boiled in a 250 cc. flask there is frequently a tendency to foam, and that unless it is watched closely the solution may boil over.

The addition of 0.6 cc. of concentrated hydrochloric acid when the potassium chloroplatinate precipitate is taken up with 6 cc. of 80 per cent alcohol is for the purpose of dissolving traces of iron and aluminum phosphates that are not completely precipitated by the addition of ammonium hydroxide in the presence of ammonium oxalate. It has been suggested that methods of precipitating these substances completely should be studied.

It is recommended[1]—

(1) That the problem of foaming during the boiling of the sample in the present official method for the determination of potash in mixed fertilizers be studied.

(2) That further study be given to the following suggested change in the last two sentences of Section **44 (a)**, page 30, "Weigh and remove the chloroplatinate precipitate by washing with hot water, using slight suction. Wash with 80 per cent alcohol three times, dry as before, and weigh (loss equals K_2PtCl_6). Calculate to K_2O."

(3) That studies be made of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the potash determination.

(4) That the words, "after 15 minutes" be added at the beginning of the sixth sentence, line 9, under **44(a)**, page 30, *Methods of Analysis A.O.A.C.*, 1935, so that the sentence will then read, "After 15 min. filter on a Gooch crucible . . . ."

REPORT ON ACID AND BASE-FORMING QUALITY OF FERTILIZERS

By L. E. HORAT (Purdue University Agricultural Experiment Station, LaFayette, Ind.), *Associate Referee*

The need and nature of further investigation of the Pierre method, *Methods of Analysis, A.O.A.C.*, 1935, 34–35, for determining the acid- and base-forming quality of fertilizers was indicated by the report of John B. Smith, the associate referee on this subject for 1935, *This Journal*, **19**, 287 (1936), and by the experiences of previous collaborators in this work.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 51 (1937).

As pointed out by Smith, the Pierre method supplies a sound basis for further work. Lack of sufficiently good agreement in results obtained in previous collaborative work, however, suggested the need for minor changes in technical details of the tentative Pierre method in order to make it applicable as a quantitative measure of acidity or basicity.

The chief sources of error appear to be the spattering of many samples during the drying preliminary to ashing and the end point of the titration. The prevention of loss by spattering is a practical problem to be solved by greater care and improved mechanical means rather than by theoretical considerations. On the other hand, improvement of the titration and end point must be considered from a theoretical as well as practical point of view.

According to Pierre,[1] "It has been shown by Britton[2] that in the titration of phosphoric acid the first hydrogen of the acid is neutralized at a pH of about 5.5. Since the transition range of methyl red is between pH 5.0 and 6.0 this indicator was selected for determining the end point of titration. That it gives the desired end point is shown by . . . equivalent basicity values of mono-, di-, and tricalcium phosphate [which] agree well with the theoretical values. The end point taken was the first change in color from reddish pink to slightly orange pink."

Smith, *This Journal*, **18**, 221–37 (1935), stated, "The first observable change with methyl red is at about pH 5; the color becomes orange at 5.5 and yellow at 6, but all of these changes are gradual, and different analysts choose different stopping points. If lower concentrations are used, smaller changes result, and it is even more difficult to duplicate the end point."

The results of field experiments with superphosphate by various workers and of pot experiments with various phosphates in soils of different degrees of acidity, by Pierre,[2] justified the selection of the mono-calcium phosphate end point, since at the average reaction value of soils of the humid regions (pH 5–6), mono-calcium phosphate was found to have no significant effect on soil acidity.

From a theoretical standpoint, the first hydrogen of phosphoric acid was shown by Britton,[3] Clark,[4] and Wendt and Clark[5] to be titrated at pH 4.5 when calcium hydroxide as well as sodium hydroxide was used. For this reason titration to an end point of pH 4.5 (the least buffered point on the curve) should give a definite and distinct color change with the proper indicator. Difficulties in titration (indicator) above pH 4.5 are due, according to Britton, and Wendt and Clark, to the instability and rearrangements of calcium phosphates produced in consequence of the titration.

The above considerations led the Associate Referee to choose brom phenol blue, which gave a more definite and distinct color change at the

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[1] *Ind. Eng. Chem. Anal, Ed.*, **5**, 229 (1933).
[2] *J. Am. Soc. Agron.*, **26**, 278 (1934).
[3] Hydrogen Ions. D. Van Nostrand Co. (1929).
[4] The Determination of Hydrogen Ions. Williams and Wilkins Co. (1929).
[5] *J. Am. Chem. Soc.*, **45**, 881 (1923).

end point (pH 4.3–4.5) than did the methyl red recommended in the original Pierre method. Comparatively small differences in results, however, were observed when brom phenol blue was used as the indicator. No doubt the slightly more basic results were due in part to the more acid end point (pH 4.5) and in part to the increased buffering effect of fertilizers high in phosphates or lime above pH 4.5–5.0.

Collaborative work was started in May, 1936, to obtain the reaction of various workers to the use of the suggested indicator (brom phenol blue) in conjunction with a more careful technic for the drying preliminary to ashing as indicated by work done in this laboratory and to learn whether more satisfactory agreement in results by various workers could thereby be attained.

Inasmuch as the samples used in previous collaborative work contained less than 16 per cent available phosphoric acid, it was thought desirable to send out some fertilizer samples higher in phosphate, with and without limestone. The following representative fertilizer samples were prepared (Table 1).

TABLE 1.—*Samples*

SAMPLE NUMBER	FORMULA	LIMESTONE ADDED	INSOLUBLE P_2O_5	TOTAL N
			per cent	*per cent*
A-1	4–24–12	yes	1.0	3.9
A-2	4–24–12	no	0.8	2.6
A-3	4–12–4	yes	1.5	4.1
A-4	4–12–4	no	1.4	3.7
A-5	2–12–6	yes	1.6	2.3
A-6	2–12–6	no	1.3	1.9
A-7	0–44–0	yes	0.4	—
A-8	0–44–0	no	0.3	—
A-9	0–20–0	yes	1.6	—
A-10	0–20–0	no	1.2	—

The acid- or base-forming qualities of the samples used were determined first by the Pierre method and later by the same method modified to include the changes mentioned, namely, more careful preliminary drying and the use of brom phenol blue indicator for titration of an aliquot part, rather than the entire sample.

MODIFIED PIERRE METHOD FOR DETERMINATION OF ACIDITY OR BASICITY OF FERTILIZERS

Follow directions of the first (Pierre) method with the following modifications:

1. REAGENTS

(b) Use a standard basic solution of 0.1 N NaOH instead of 1 N NaOH.

(c) In place of methyl red use brom phenol blue indicator, 0.4% solution. Dissolve 1 gram of the dye in 250 cc. of 50% ethyl alcohol.

(d) After boiling the blank solution to remove CO_2, proceed as follows:

Cool, and make to a volume of 250 cc. Titrate an aliquot part, 100 cc., with 0.1 N NaOH to a grey magenta color. The change from yellowish magenta to grey magenta color (pH 4.3–pH 4.5) is observed best in a thin layer of solution by transmitted light. This titration value is the blank for the solution.

2. DETERMINATION

Instead of evaporating the Na_2CO_3 solution of the sample on a sand bath proceed as follows: Place on a steam bath and evaporate as completely as possible. Remove, break the film on the surface by rotating the beaker, and dry in an oven heated to approximately 120–125°C. until charring has begun. Place the beaker in a furnace heated to approximately 250°C. and proceed as directed in the original method until the acidified ash solution has been filtered. Cool the entire clear filtrate and make to a volume of 250 cc. To an aliquot part, 100 cc., add 10 drops of brom phenol blue indicator and titrate to a grey magenta color (pH 4.3–4.5) with 0.1 N NaOH.

Subtract algebraically the cc. of 0.1 N NaOH used in the titration of the 100 cc. of the fertilizer from the cc. of 0.1 N NaOH used in the titration of the same aliquot part of the blank. Divide this difference by four to convert to cc. of N NaOH required by the entire sample used. For a 1 gram sample, multiply the result by 100; 0.5 gram sample, by 200; 0.25 gram sample, by 400. From this point proceed as directed in the original Pierre method.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers*

SAMPLE A-1, 4-24-12, LIMESTONE ADDED			SAMPLE A-2, 4-24-12, NO LIMESTONE ADDED		
NUMBER OF COLLABORATOR	PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)		PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)
1	17A	1A		199A	181A
2	76B	84B		163A	118A
3	38B	51B		191A	166A
4	54B	84B		120A	122A
5	39B	28B		139A	149A
6	12B	29B		176A	158A
7	23B	86B		165A	160A
8	43B	48B		186A	165A
9	13B	26B		143A	140A
10	48A*	52B		205A	128A
11	53B	58B		101A	106A
12	78B	44B		126A	139A
13	6B	67B		244A*	155A
14	90B	97B		173A	163A
15	48B	40B		160A	165A
16	95B	115B		125A	88A
17	68B	67B		120A	140A
18	33B	33B		175A	150A
19	37B	72B		150A	136A
20	12B	41B		215A	194A
21	33B	66B		175A	154A
22	13B	55A*		210A	281A*
23	36B	66B		185A	190A
24	28B	95B		158A	132A
25	4B	50B		197A	146A
26	56B	23B		145A	173A
Average Basicity (lbs. $CaCO_3$ per ton)	38	57	Average Acidity (lbs. $CaCO_3$ per ton)	165	149
Av. Deviation	23	16	Av. Deviation	25	20
Max. Deviation	57	58	Max. Deviation	64	61

* Omitted from average.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers (Continued)*

	SAMPLE A-3, 4-12-4, LIMESTONE ADDED		SAMPLE A-4, 4-12-4, NO LIMESTONE ADDED	
NUMBER OF COLLABORATOR	PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)	PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)
1	138A	134A	271A	283A
2	131A	72A	282A	226A
3	123A	107A	281A	255A
4	114A	71A	246A	237A
5	105A	100A	259A	256A
6	138A	116A	300A	267A
7	109A	103A	332A	268A
8	122A	94A	259A	249A
9	89A	94A	247A	259A
10	171A	91A	304A	246A
11	108A	105A	279A	260A
12	99A	98A	259A	251A
13	148A	120A	306A	265A
14	118A	118A	267A	262A
15	126A	123A	282A	273A
16	142A	99A	287A	218A
17	89A	85A	252A	248A
18	134A	109A	277A	272A
19	144A	124A	278A	260A
20	122A	116A	276A	250A
21	124A	100A	262A	252A
22	157A	151A	299A	309A
23	114A	116A	262A	247A
24	169A	119A	313A	259A
25	104A	79A	271A	225A
26	99A	111A	216A	253A
Average Acidity (lbs. $CaCO_3$ per ton)	125	104	268	254
Av. Deviation	18	15	20	11
Max. Deviation	46	47	64	55

	SAMPLE A-5, 2-12-6, LIMESTONE ADDED		SAMPLE A-6, 2-12-6, NO LIMESTONE ADDED	
1	481B	488B	118A	114A
2	490B	536B	98A	126A
3	493B	517B	115A	85A
4	478B	533B	99A	79A
5	535B	530B	117A	96A
6	480B	535B	137A	105A
7	464B	502B	121A	140A
8	493B	563B	104A	78A
9	554B	575B*	90A	93A
10	407B	509B	125A	80A
11	473B	497B	90A	92A

* Omitted from average.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers* (*Continued*)

	SAMPLE A-5, 2-12-6, LIMESTONE ADDED		NO SAMPLE A-6, 2-12-6, LIMESTONE ADDED	
NUMBER OF COLLABORATOR	PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)	PIERRE METHOD (TENTATIVE A.O.A C.)	PIERRE METHOD (MODIFIED)
12	508B	501B	130A	77A
13	396B	502B	147A	94A
14	439B	493B	100A	90A
15	485B	499B	90A	98A
16	462B	518B	94A	70A
17	515B	564B	89A	85A
18	483B	523B	119A	119A
19	460B	493B	101A	79A
20	513B	533B	94A	103A
21	456B	538B	115A	100A
22	439B	523B	101A	141A
23	541B	511B	105A	113A
24	387B*	456B*	136A	65A
25	498B	570B	101A	62A
26	579B*	537B	77A	92A

Average Basicity (lbs. $CaCO_3$ per ton)	481	501	Average Acidity (lbs. $CaCO_3$ per ton)	108	95
Av. Deviation	31	23	Av. Deviation	14	16
Max. Deviation	81	69	Max. Deviation	39	46

	SAMPLE A-7, 0-44-0, LIMESTONE ADDED		SAMPLE A-8, 0-44-0, NO LIMESTONE ADDED	
1	92B	110B	83A	67A
2	162B	152B	35A	37A
3	104B	128B	58A	56A
4	134B	147B	28A	55A
5	131B	136B	44A	37A
6	78B	134B	116A	30A
7	159B	136B	89A	50A
8	100B	134B	90A	54A
9	129B	149B	4A	24A
10	64B	181B	117A	13A
11	149B	147B	28A	38A
12	183B	160B	93A	42A
13	35B	144B	167A	48A
14	113B	148B	39A	14A
15	149B	166B	49A	36A
16	139B	183B	9B	23B
17	164B	163B	8A	6A
18	99B	134B	88A	43A
19	133B	165B	39A	35A
20	91B	110B	46A	63A
21	129B	164B	59A	38A
22	95B	117B	198A*	73A

* Omitted from average.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers* (*Continued*)

	SAMPLE A-7, 0-44-0, LIMESTONE ADDED			SAMPLE A-8, 0-44-0, NO LIMESTONE ADDED	
NUMBER OF COLLABORATOR	PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)		PIERRE METHOD (TENTATIVE A.O.A.C.)	PIERRE METHOD (MODIFIED)
23	99B	131B		22A	77A
24	83B	166B		75A	45A
25	79B	173B		84A	10A
26	186B	168B		10A	16B
Average Basicity (lbs. $CaCO_3$ per ton)	118	148	Average Acidity (lbs. $CaCO_3$ per ton)	56	37
Av. Deviation	32	17	Av. Deviation	35	19
Max. Deviation	83	38	Max. Deviation	111	60

	SAMPLE A-9, 0-20-0, LIMESTONE ADDED			SAMPLE A-10, 0-20-0, NO LIMESTONE ADDED	
1	323B	328B		32A	15A
2	312B	338B		43A	16B
3	325B	341B		19A	2B
4	330B	340B		16B	19B
5	316B	329B		20A	18A
6	315B	366B		66A	1A
7	320B	355B		73A	75A
8	333B	381B		25A	5B
9	393B	390B		14A	9A
10	238B	354B		82A	18B
11	305B	343B		32A	11A
12	345B	361B		14A	2A
13	213B	334B		111A	10A
14	329B	349B		21A	9A
15	355B	420B		1A	14B
16	324B	377B		19A	18B
17	375B	381B		4B	9B
18	340B	385B		44A	14A
19	189B*	235B*		21A	6A
20	305B	297B		6A	46A
21	330B	367B		1B	6B
22	299B	379B		23A	31A
23	305B	359B		21A	5A
24	266B	344B		70A	17B
25	339B	435B		6A	46B
26	445B*	432B*		33B	16B
Average Basicity (lbs. $CaCO_3$ per ton)	318	361	Average Acidity (lbs. $CaCO_3$ per ton)	27	3
Av. Deviation	26	22	Av. Deviation	22	17
Max. Deviation	105	74	Max. Deviation	85	72

* Omitted from average.

TABLE 3.—*Summary of collaborative results*

SAMPLE NUMBER	ANALYSIS	LIMESTONE ADDED	$CaCO_3$ EQUIVALENT IN POUNDS PER TON OF FERTILIZER						AVERAGE DIFFERENCE* BETWEEN METHODS
			PIERRE METHOD (TENT. A. O. A. C.)			PIERRE METHOD (MODIFIED)			
			AVERAGE VALUE	DEV. FROM AV. VALUE		AVERAGE VALUE	DEV. FROM AV. VALUE		
				AVERAGE	MAXIMUM		AVERAGE	MAXIMUM	
A- 1	4-24-12	Yes	38-B	23	57	57-B	16	58	19
A- 2	4-24-12	No	165-A	25	64	149-A	20	61	16
A- 3	4-12-4	Yes	125-A	18	46	104-A	15	47	21
A- 4	4-12-4	No	268-A	20	64	254-A	11	55	14
A- 5	2-12-6	Yes	481-B	31	81	501-B	23	69	20
A- 6	2-12-6	No	108-A	14	39	95-A	16	46	13
A- 7	0-44-0	Yes	118-B	32	83	148-B	17	38	30
A- 8	0-44-0	No	56-A	35	111	37-A	19	60	19
A- 9	0-20-0	Yes	318-B	26	105	361-B	22	74	43
A-10	0-20-0	No	27-A	22	85	3-A	17	72	24
Grand Averages (for all samples)				24.4			17.6		21.9

* Expressed in pounds $CaCO_3$ per ton
In all cases results were more basic by modified Pierre method than by original Pierre method.

RESULTS OF COLLABORATORS

In Table 2 the equivalent acidities or basicities for each sample, as determined by each collaborator by both methods, are given in pounds of calcium carbonate per ton of fertilizer. The results represent the average values of from two to ten separate determinations. A summary of the results, Table 3, shows that in every case values of slightly greater acidity were obtained by the original Pierre method than by the modified method.

A comparison of results for the referee samples obtained by the writer by electrometric titration and by indicator titrations are recorded in Table 4, while similar data submitted by John B. Smith are given in Tables 5 and 6.

TABLE 4.—*Values* obtained by electrometric titrations compared with those obtained by indicator titrations*

SAMPLE NUMBER	ELECTROMETRIC TITRATION TO *pH* 4.4	ELECTROMETRIC TITRATION TO *pH* 5.0	DIFFERENCE†	INDICATOR TITRATION WITH B.P.B. (*pH* 4.4)	INDICATOR TITRATION WITH M.R. (*pH* 5.0–5.1)	DIFFERENCE†
A- 1	62-B	43-B	19	68-B	63-B	5
A- 2	161-A	175-A	14	170-A	185-A	15
A- 3	102-A	110-A	8	104-A	104-A	0
A- 4	242-A	249-A	7	247-A	252-A	5
A- 5	531-B	503-B	28	516-B	508-B	8
A- 6	96-A	108-A	12	102-A	105-A	3
A- 7	108-B	96-B	12	137-B	124-B	13
A- 8	41-A	78-A	37	48-A	54-A	6
A- 9	334-B	307-B	27	342-B	305-B	37
A-10	42-A	60-A	18	4-A	8-A	4

* All values expressed in pounds $CaCO_3$ per ton. All titrations made on aliquot parts of the same solution. All *p*H determinations made with quinhydrone electrode. Quinhydrone electrode was checked against standard buffer, which was checked against glass electrode (Beckman).

† Values represent pounds $CaCO_3$ per ton greater basicity when titrated to more acid *p*H or with brom phenol blue.

TABLE 5.—pH *of end points from titration by quinhydrone electrode*

SAMPLE NO.	METHYL RED	BROM PHENOL BLUE
A- 1	5.13	4.29
A- 2	4.90	4.30
A- 3	4.83	4.46
A- 4	4.90	4.34
A- 5	4.93	4.49
A- 6	4.95	4.33
A- 7	4.90	4.49
A- 8	4.97	4.44
A- 9	4.93	4.60
A-10	4.76	4.37

* Data submitted by John B. Smith.

TABLE 6.*—*Effect of the two end points on apparent basicity of the ash. Aliquot parts of 100 cc. from the same solution titrated with 0.1 N* NaOH, *one to pH 4.9–5.1 with methyl red, and the other to* pH *4.3–4.5 with brom phenol blue and verified with the quinhydrone electrode*

SAMPLE NO.	GREATER BASICITY WITH BROM PHENOL BLUE IN LBS. $CaCO_3$ EQUIVALENT PER TON	SAMPLE NO.	
A-1	78	A- 6	41
A-2	45	A- 7	68
A-3	33	A- 8	82
A-4	44	A- 9	62
A-5	44	A-10	42

* Data submitted by John B. Smith.

COLLABORATORS*

1. H. B. Siems and R. C. Koch, Chicago, Ill.
2. H. C. Batton, Baltimore, Md.
3. H. D. Haskins and J. W. Kuzmeski, Amherst, Mass.
4. H. C. Moore and R. D. Caldwell, Atlanta, Ga.
5. C. A. Butt and C. M. Cartledge, East Point, Ga.
6. R. Neu, Jacksonville, Fla.
7. G. S. Fraps and T. L. Ogier, College Station, Texas.
8. C. P. Coleman, Tallahassee, Fla.
9. C. R. Byers, Carteret, N. J.
10. E. M. Bailey and W. T. Mathis, New Haven, Conn.
11. F. B. Carpenter, H. L. Moxon, and R. O. Powell, Richmond, Va.
12. R. L. Jones, Chicago Heights, Ill.
13. H. A. Davis, Durham, N. H.
14. C. L. Hare and T. H. Burton, Auburn, Ala.
15. S. M. Wilson and R. D. Dent, Baltimore, Md.
16. B. F. Robertson and J. T. Foy, Clemson College, S. C.
17. C. C. Howes and Mary C. Fox, Baltimore, Md.
18. E. W. Magruder and W. A. Ryder, Norfolk, Va.
19. W. F. Hand and M. P. Etheredge, State College, Miss.
20. W. R. Austin, Nashville, Tenn.
21. L. E. Horat, Lafayette, Ind.
22. L. D. Haigh and E. W. Cowan, Columbia, Mo.
23. A. P. Bell, Lafayette, Ind.
24. John B. Smith, D. R. Willard, and Thomas Wright, Jr., Kingston, R. I.
25. Robt. P. Thornton, Tampa, Fla.
26. T. B. Leith, Charleston, W. Va.

COMMENTS OF COLLABORATORS

In response to a questionnaire submitted to each collaborator, the following observations, opinions, or preferences in reference to certain details of technic were obtained.

* Of the 26 collaborators submitting reports, 13 are from the laboratories of fertilizer companies, 12 are from Experiment Stations or similar institutions, and one is from a commercial laboratory.

Two-thirds of the collaborators used Pyrex beakers, while one-half preferred Pyrex beakers of 150 cc. capacity. Silica, porcelain, and sillimanite beakers ranked next in the order named.

One-third of the collaborators observed at least slight spattering during the preliminary drying on the sand bath. Three collaborators reported spattering in the oven at 125°, and one observed it during ashing in the furnace at 600.° For the preliminary drying preference was almost equally divided for an oven at 125°, sand bath, and hot plate, in the respective ratio of 5, 5, 4.

For the titration with brom phenol blue the need for more indicator was reported by five collaborators. A sharp and distinct end point during titration with methyl red as indicator was reported in six cases; with brom phenol blue as indicator the same was reported in eleven cases. A poor end point for both indicators was reported by eight collaborators.

Cloudiness or precipitation during titration was noted in 15 cases when methyl red was used and three cases with brom phenol blue. Interference with the observation of the end point due to cloudiness or precipitation was reported in nine cases when methyl red was used, and in two cases with brom phenol blue. Fifteen of the collaborators indicated a preference for use of 0.5 *N* NaOH and the entire filtrate, when titrating with brom phenol blue.

In addition to the summarized replies given, several collaborators made more lengthy comments regarding the two methods. In four of these cases a decided preference for the original Pierre method was indicated, while eight collaborators reported a decided preference for the use of brom phenol blue as the indicator.

ACKNOWLEDGMENT

The writer wishes to express his indebtedness to H. R. Kraybill and all the collaborators and his gratitude for their valuable comments and splendid cooperation. The many suggestions of the former associate referee, John B. Smith, were especially helpful and greatly appreciated.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modified method submitted by the associate referee for the determination of acid- and base-forming quality of fertilizer be further studied.

(2) That the present tentative method for the determination of acid- and base-forming quality of fertilizer be studied in regard to the following proposed changes: (a) Substituting 0.5 *N* NaOH in the titration in place of 1.0 *N* NaOH; (b) minimizing the loss by spattering; and (c) eliminat-

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 51 (1937).

ing water-insoluble material coarser than 20 mesh by wet sieving before the method is applied.

(3) That the basicity of the phosphate rock and other factors that affect the method be studied further.

The paper, entitled "Effect of Removing Water-soluble Compounds Prior to Determination of Citrate-insoluble Phosphorus in Fertilizers," presented by K. D. Jacob and T. H. Tremearne is published in *This Journal*, p. 277

The paper, entitled "Determination of Urea Nitrogen in Fertilizer Mixtures," presented by J. Y. Zee and R. O. E. Davis, was published in *This Journal*, **20**, 104 (1937).

The paper, entitled "Use of Silica Dishes in the Official Method for the Determination of Potash in Fertilizers," presented by H. R. Allen and Lelah Gault," was published in *This Journal*, **20**, 101 (1937).

OMISSION

The recommendation of the Committee on Recommendations of Referees that par. 3, p. 497, of Chap. XXXV, *Methods of Analysis, A.O.A.C.*, 1935, be changed to read "Total Solids—Tentative. Proceed as directed under **16**."

ASSOCIATE REFEREES

Louis Ehrenfeld, Wahl-Henius Institute, Chicago, Ill., was appointed Associate Referree on Diastatic Power in Malt.

The Associate Referee on Mineral Mixed Feeds should read Alfred T. Perkins, not H. E. Perkins, as given in the February Number of *This Journal*, p. 4.

The sudden death of George L. Bidwell of the Food Division, U. S. Food and Drug Administration, on February 20th, was a great shock to his many friends and coworkers in the Department of Agriculture and in the Association of Official Agricultural Chemists, where he had served in many offices.

A biographical sketch of his life and work will appear in a later number of *This Journal*.

METHODS OF ANALYSIS, A.O.A.C., 4th EDITION, 1935

ERRATA AND REFEREES' EMENDATIONS*

Page	Section	
18,	**5**, note under Hdg.	Delete first word, "Not."
30,	**44(a)**, line 9	Insert "After 15 min." before "Filter."
126,	**16**, line 11	Change "$KClO_3$" to "$KClO_4$."
135,	**46**	Change "39" to "38."
149,	**6**, line 5	Change "5" to "5(a)."
	8, line 2	Enclose "$A \times 2.0665+E$" in parentheses.
	last line	Change "6" to "7(a) or (c)."
150,	**10**, line 1	Delete "to remove CO_2."
	18, line 2	Change "evaporate to dryness" to "concentrate to sirupy consistency."
151,	**20**, 3rd line from bottom	Change "thymolphthalein" to "phenolphthalein."
152,	**21**, line 1	Delete "necessarily."
	27	Change to "Place a 100 cc sample in a Pt dish, add 200 cc of a 5% soln of Na_2CO_3 and proceed as directed under **XII, 34–37.**
	28, line 4	Change "19" to "23" and add "*See also* **XVI, 25.**"
160,	**51**, line 6	Change "prepared" to "finely ground."
161,	**51**, line 3	Change "inversion" to "conversion."
	55, line 11	Change "inverted" to "converted."
168,	**30(c)**	Change "sulfonate" to "disulfonate."
175,	**24**, note, last line	Change "Table **24**" to "**XLII**, Table **20.**"
195,	**35, 36**	Change "11" to "12."
212,	**29**, line 2	After "add" insert "30 cc. of C_2H_5OH and."
	line 5	After "separatory funnel" add "(glass stopcock lubricated with water)."
305,	**28**	Change "72" to "79."
338,	**19**, line 6	Change "(h)" to "(f)."
347,	**44**, line 8	Change "(pH 3.0–4.4)" to "(pH 2.5–3.0)."
	line 9	Change "20–30 cc" to "10 cc."
348,	47, line 3	Add "2–4" hrs." to sentence in parentheses.
365,	**41**, line 2	Change "**32, 33**" to "**34, 35.**"
378, 379,	**13(j)** and **(p)**	Insert "phosphate-free" before "KCN."
423,	line 2	Add "If the melting point of the glycerides plus twice the difference between the melting point of the glycerides and the melting point of the fatty acids, is less than 73°, the lard is regarded as adulterated."
439,	**29**, last line	Delete subscript "$_5$."
460,	**79**, last line	Change "**26**" to "**27.**"
463,	**5**	Transpose Hdg. "By Means of a Spindle, etc." and the preceding note in parentheses.
464,	**6**, line 4	Change "LXII" to "XLII."
496,	Ref. 35	Add "Trans. Roy. Soc. Can., 1919, Sec. III, 221."
	Ref. 38	Add "J. Ind. Eng. Chem., **5**, 741 (1913); **8**, 331 (1916); and J. Assoc. Official Agr. Chem., **4**, 435 (1931), and delete "J. Am. Chem. Soc., **28**, 435 (1906)."
500,	**22**, line 2	Change "**33** and **35**" to "**34.**"
558,	**44**, line 13	Change "0.0043" to "0.00403."
619,	last column, line 12	Change "0.80" to "0.08."
626,	column 7, line 17	Change "29.6" to "26.9."

* Reprinted from *J. Assoc. Official Agr. Chem.*, May, 1937. *See also* report on "Changes in Methods" in the February number of each year.

CONTRIBUTED PAPERS

EFFECT OF REMOVING WATER-SOLUBLE COMPOUNDS PRIOR TO DETERMINATION OF CITRATE-INSOLUBLE PHOSPHORUS IN FERTILIZERS*

By K. D. Jacob and T. H. Tremearne
(Fertilizer Research Division, Bureau of Chemistry and Soils, Washington, D.C.)

The official method for the determination of citrate-insoluble phosphorus in *acidulated samples* (*15*) directs that water-soluble phosphorus be removed, prior to the citrate digestion, by washing the charge on a 9 cm. filter with successive small portions of water until the filtrate measures about 250 ml. In 1923 Austin (*1*) reported data that indicated the importance of washing superphosphate and mixed fertilizers with water to the volume of 250 ml. as directed in the official method. In the case of superphosphate the results obtained for citrate-insoluble P_2O_5 in charges that were washed with successive small portions of water to a volume of 250 ml. were 0.15–0.30 per cent lower than those obtained when unwashed charges were digested directly with citrate solution.

At the time that Austin's work was done the official method specified the use of a 2 gram charge, but in 1931 the weight of the charge was officially changed from 2 grams to 1 gram (*10*, *16*, *17*). Data reported by Jacob, Rader, and Tremearne (*8*) on four ordinary and double superphosphates indicate that with 1 gram charges of such materials it makes little difference in the results whether the water-soluble compounds are removed before the citrate digestion or the entire charge is digested without preliminary washing. Inasmuch as the time required for the analysis would be reduced considerably by omitting the preliminary washing, it seemed desirable to make a further study of the effect of the presence of water-soluble compounds on the results for citrate-insoluble phosphorus. Accordingly, data were obtained on 43 samples of superphosphates, ammoniated superphosphates, limed superphosphates, wet-mixed base goods, and complete fertilizer mixtures.

MATERIALS AND ANALYTICAL METHODS

Ordinary superphosphates Nos. 1500 and 1509 and the complete fertilizer mixtures were made in the late winter and early spring of 1936. The other materials were made during the period 1928 to 1935, principally before 1935. The ammoniated ordinary and double superphosphates were made with either straight ammonia liquor or anhydrous ammonia. In the preparation of the laboratory-mixed fertilizers (Table 1) anhydrous ammonia was added at the time the mixtures were made.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 30 to December 2, 1936.

TABLE 1.—*Formulas of laboratory-mixed fertilizers*
(Pounds of material per 2000 pounds of mixture)

MATERIAL	MIXTURE					
	NO. 1525, 4-8-7	NO. 1527, 4-8-8	NO. 1523, 5-10-5	NO. 1524, 5-10-5	NO. 1526, 4-16-4	NO. 1522, 5-20-5
Anhydrous ammonia	—	—	30	30	37	60
Ammonium sulfate	195.0	390	288	192	146	192
Sodium nitrate	123.4	—	92	61	123	61
Cottonseed meal	—	—	—	347	—	—
Tankage	227.2	—	—	—	—	—
Superphosphate	794.0	794	816	816	1225	640
Double superphosphate	—	—	86	86	157	588
Potassium chloride	269.8	276	173	173	138	173
Kieserite	95.0	—	—	—	50	—
Dolomitic limestone	230.0	—	340	286	124	286
Sand	65.6	540	175	9	—	—

Except as it is stated otherwise, the official methods (*15*) for total phosphorus, water-soluble phosphorus, and for citrate-insoluble phosphorus in *acidulated samples* were carefully followed. Water-soluble phosphorus was removed by washing the charge on a 9 cm. paper (Whatman's No. 1) with 11 ml. portions of cold water and allowing thorough gravity draining between washings. The water-insoluble residue was digested with citrate immediately. When the preliminary washing with water was omitted the charge was folded into a 9 cm. paper (Whatman's No. 1) and dropped into the citrate solution. The citrate extract was filtered through a double thickness of Whatman's No. 5 paper. Phosphorus was determined by the volumetric method with stirring of the yellow precipitate at room temperature.

The results reported herein are the averages of duplicate determinations.

EFFECT OF REMOVING WATER-SOLUBLE COMPOUNDS

The results (detailed in Table 2 and summarized in Table 3) for citrate-insoluble phosphorus in 1 gram samples are, with three exceptions, higher on the unwashed charges than on the washed ones. The differences between the results on washed and unwashed charges range from 0.02 to 3.34 per cent of P_2O_5. In general the differences are smallest (frequently negligible) with ordinary and double superphosphates and largest with heavily limed superphosphate and with ammoniated ordinary superphosphate.

With the possible exception of the ammoniated ordinary superphosphates, the limed superphosphates, and the wet-mixed base goods, there appears to be no consistent relationship between the effect of washing the charge prior to the citrate digestion and either the nature of the material

TABLE 2.—*Effect of removing water-soluble compounds prior to determination of citrate-insoluble phosphorus*
(Analyses made on 1 gram charges)

SAMPLE	MATERIAL	FINENESS OF SAMPLE	TOTAL P_2O_5	WATER-SOLUBLE P_2O_5	CITRATE-SOLUBLE P_2O_5 IN WASHED SAMPLE[a]	CITRATE-INSOLUBLE P_2O_5		
						UNWASHED SAMPLE	WASHED SAMPLE[a]	DIFFERENCE[b]
		mesh	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1060	Superphosphate[c,d]	−40	20.75	17.38	2.58	0.93	0.79	0.14
1315	Superphosphate[c]	−20	19.20	16.70	2.42	0.12	0.08	0.04
1370	Superphosphate[c,d]	−20	21.73	18.32	2.84	0.73	0.57	0.16
1378	Superphosphate[c,e]	−20	20.16	12.63	3.70	4.34	3.83	0.51
1402	Superphosphate[c,f]	−20	20.48	18.50	1.65	0.61	0.33	0.28
1403	Superphosphate[c,g]	−40	20.56	20.30	0.11	0.27	0.15	0.12
1414	Superphosphate[c,f]	−20	17.57	16.18	1.31	0.13	0.08	0.05
1509	Superphosphate[c]	−40	16.48	14.68	1.73	0.22	0.07	0.15
1066	Superphosphate[h]	−20	19.88	14.14	5.65	0.15	0.09	0.06
1087	Superphosphate[h,i]	−40	21.97	19.09	2.09	0.87	0.79	0.08
1170	Superphosphate[h]	−80	20.06	15.40	4.62	0.12	0.04	0.08
1171	Superphosphate[h,j]	−80	18.48	10.05	8.32	0.32	0.11	0.21
1316	Superphosphate[h]	−20	18.96	12.30	6.57	0.14	0.09	0.05
1500	Superphosphate[h]	−20	19.22	14.60	4.48	0.22	0.14	0.08
1486	Superphosphate[c,k]	−20	32.50	28.05	4.28	0.24	0.17	0.07
1337	Double superphosphate[c,l]	−20	46.40	38.83	7.38	0.49	0.19	0.30
1338	Double superphosphate[c,l]	−20	44.85	38.00	6.77	0.29	0.08	0.21
1361	Double superphosphate[c,l]	−20	49.27	41.63	7.59	0.07	0.05	0.02
1059	Double superphosphate[h,l]	−20	43.90	29.90	13.02	0.88	0.98	−0.10
1362	Double superphosphate[h,m]	−20	48.37	42.68	5.27	0.52	0.42	0.10
1415	Double superphosphate[h,n]	−40	48.66	37.90	8.40	2.44	2.36	0.08
1481	Double superphosphate[h,l]	−20	44.31	31.20	12.54	0.81	0.57	0.24
1061	Double superphosphate[o,l,p]	−40	45.58	36.80	5.55	3.69	3.23	0.46
1368	Double superphosphate[o,l,p]	−20	47.87	41.05	3.93	3.11	2.89	0.22

TABLE 2.—*Continued*

SAMPLE	MATERIAL	FINENESS OF SAMPLE	TOTAL P_2O_5	WATER-SOLUBLE P_2O_5	CITRATE-SOLUBLE P_2O_5 IN WASHED SAMPLE[a]	CITRATE-INSOLUBLE P_2O_5		
						UNWASHED SAMPLE	WASHED SAMPLE[a]	DIFFERENCE[b]
		mesh	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1372	Double superphosphate[o,l]	−20	47.80	41.43	5.34	1.11	1.03	0.08
1103	Limed superphosphate[c,q]	−100	15.26	2.40	11.25	1.69	1.61	0.08
1185	Limed superphosphate[c,r]	−80	17.05	0.69	10.94	8.76	5.42	3.34
1168	Ammoniated superphosphate,[c] 8.7% NH_3	−40	19.33	0.32	11.37	8.61	7.64	0.97
1169	Ammoniated superphosphate,[c] 2.5% NH_3	−40	20.35	12.40	6.25	2.06	1.70	0.36
1175	Ammoniated superphosphate,[c] 5.4% NH_3	−40	19.22	7.63	10.10	2.55	1.49	1.06
1177	Ammoniated superphosphate,[h] 4.8% NH_3	−40	19.00	9.85	6.55	3.22	2.60	0.62
1517	Ammoniated double superphosphate,[c] 1.4% NH_3	−40	49.00	36.29	12.60	0.14	0.11	0.03
1518	Ammoniated double superphosphate,[c] 6.0% NH_3	−40	48.31	27.23	18.88	2.43	2.20	0.23
1520	Ammoniated double superphosphate,[c] 10.0% NH_3	−40	45.94	28.09	15.38	2.12	2.47	−0.35
1104	Wet-mixed base goods	−20	12.38	9.87	1.69	1.26	0.82	0.44
1105	Wet-mixed base goods	−20	9.29	5.41	0.90	3.36	2.98	0.38
1508	Mixed fertilizer,[s] 4-8-4	−40	9.35	3.07	5.68	0.68	0.60	0.08
1525	Mixed fertilizer,[t] 4-8-7	−40	9.03	7.00	1.84	0.73	0.19	0.54
1527	Mixed fertilizer,[t] 4-8-8	−40	8.71	7.34	1.32	0.15	0.05	0.10
1523	Mixed fertilizer,[t] 5-10-5	−40	10.48	5.10	5.34	0.30	0.04	0.26
1524	Mixed fertilizer,[t] 5-10-5	−40	11.29	5.44	5.65	0.44	0.20	0.24
1526	Mixed fertilizer,[t] 4-16-4	−40	16.75	7.95	8.68	0.25	0.12	0.13
1522	Mixed fertilizer,[t] 5-20-5	−40	21.15	10.00	10.69	0.38	0.46	−0.08

[a] Washed on a 9 cm. paper by gravity filtration to a total volume of 225 ml.; approximately 11 ml. of cold water used at each washing.
[b] Insoluble P_2O_5 in unwashed sample minus insoluble P_2O_5 in washed sample.
[c] Made from Florida pebble phosphate.
[d] Oberphos.
[e] A special commercial material prepared by using insufficient H_2SO_4 to convert all the P_2O_5 into monocalcium phosphate.
[f] Made with sludge H_2SO_4 from petroleum refinery.
[g] Made with sludge H_2SO_4 that had been treated by a special process.
[h] Made from Tennessee brown-rock phosphate.
[i] Artificially dried "den" superphosphate.
[j] Experimentally prepared from phosphate rock containing 8.15% $Fe_2O_3+Al_2O_3$.
[k] Commercial material prepared by granulating a mixture of ordinary superphosphate and double superphosphate.
[l] Phosphoric acid was made by H_2SO_4 process.
[m] Phosphoric acid was made by blast furnace process.
[n] Phosphoric acid was made by electric furnace process.
[o] Made from Idaho phosphate.
[p] A small quantity of lime was added as a conditioner.
[q] Commercial material prepared by mixing 1 part of dolomitic limestone with 4.33 parts of superphosphate.
[r] Experimental material prepared by mixing slurries of 1 part of $Ca(OH)_2$ and 5 parts of superphosphate, allowing to stand 1 week, and drying at 45° C.
[s] Commercial mixture.
[t] Laboratory mixture. See Table 1.

TABLE 3.—*Summary of results on effect of removing water-soluble compounds prior to determination of citrate-insoluble phosphorus*
(Analyses made on 1 gram charges)

MATERIAL	SAMPLES ANALYZED	DIFFERENCE BETWEEN RESULTS FOR CITRATE-INSOLUBLE P_2O_5 IN UNWASHED AND WASHED CHARGES	
		RANGE[a]	AVERAGE[a]
		per cent	*per cent*
Ordinary superphosphate, all samples	14	0.04–0.51[b]	0.14
Ordinary superphosphate, Florida pebble	8	0.04–0.51[c]	0.18
Ordinary superphosphate, Tennessee brown rock	6	0.05–0.21	0.09
Double superphosphate, all samples[d]	11	0.02–0.46	0.17
Double superphosphate, Florida pebble[d]	4	0.02–0.30	0.15
Double superphosphate, Tennessee brown rock	4	0.08–0.24	0.13
Double superphosphate, Idaho rock	3	0.08–0.46	0.25
Limed superphosphate	2	0.08–3.34	1.71
Ammoniated ordinary superphosphate	4	0.36–1.06	0.75
Ammoniated double superphosphate	3	0.03–0.35	0.20
Wet-mixed base goods	2	0.38–0.44	0.41
Mixed fertilizer	7	0.08–0.54	0.20

[a] Disregarding signs.
[b] Excluding No. 1378, which was made with a deficiency of H_2SO_4 and is exceptionally high in citrate-insoluble P_2O_5, the range is 0.04–0.28% and the average 0.11%.
[c] Excluding No. 1378 the range is 0.04–0.28% and the average 0.13.%.
[d] Including No. 1486, a mixture of ordinary and double superphosphate.

or its content of total, water-soluble or citrate-soluble phosphorus. In the case of the ammoniated ordinary superphosphates, the differences between the results for citrate-insoluble phosphorus in unwashed and washed charges, respectively, usually increase with the ammonia content of the material or, correspondingly, with the decrease in water-soluble phosphorus and increase in citrate-soluble phosphorus. No definite conclusions can be drawn from the results obtained on the limed superphosphates, because No. 1103 was prepared with dolomitic limestone whereas No. 1185 was prepared with calcium hydroxide. The data on wet-mixed base goods are too meager to justify the drawing of definite conclusions. With the laboratory-mixed fertilizers there appears to be no relationship between the composition of the mixture and the effect of washing the sample prior to the citrate digestion.

In general, varying the weight of the sample between the limits 0.5 gram and 2 grams does not have a uniform effect on the differences between the results for citrate-insoluble phosphorus in unwashed and washed charges (Table 4).

EFFECT OF AMOUNT OF WASHING PRIOR TO CITRATE DIGESTION

Austin (*1*) reported that the percentages of citrate-insoluble phosphorus in 2 gram charges of superphosphates and mixed fertilizers, which were

TABLE 4.—*Effect of weight of sample on results for citrate-insoluble phosphorus in washed and unwashed charges*

(Results expressed in percentage)

SAMPLE	MATERIAL	0.5 GRAM SAMPLE				1.0 GRAM SAMPLE				2.0 GRAM SAMPLE			
		WATER-SOLUBLE P_2O_5	CITRATE-INSOLUBLE P_2O_5			WATER-SOLUBLE P_2O_5	CITRATE-INSOLUBLE P_2O_5			WATER-SOLUBLE P_2O_5	CITRATE-INSOLUBLE P_2O_5		
			UN-WASHED	WASHED[a]	DIFFERENCE[b]		UN-WASHED	WASHED[a]	DIFFERENCE[b]		UN-WASHED	WASHED[a]	DIFFERENCE[b]
1315	Superphosphate	17.50	0.09	0.06	0.03	16.70	0.12	0.08	0.04	16.48	0.09	0.07	0.02
1378	Superphosphate	12.64	3.89	3.10	0.79	12.63	4.34	3.83	0.51	12.15	4.52	4.09	0.43
1402	Superphosphate	18.45	0.42	0.23	0.19	18.50	0.61	0.33	0.28	18.31	0.59	0.47	0.12
1500	Superphosphate	15.18	0.13	0.03	0.10	14.60	0.22	0.14	0.08	14.48	0.24	0.13	0.11
1361	Double superphosphate	41.65	0.06	0.08	−0.02	41.63	0.07	0.05	0.02	41.45	0.08	0.06	0.02
1368	Double superphosphate	41.23	3.05	2.38	0.67	41.05	3.11	2.89	0.22	39.78	3.17	3.10	0.07
1481	Double superphosphate	31.30	0.72	0.56	0.16	31.20	0.81	0.57	0.24	30.20	0.90	0.67	0.23
1103	Limed superphosphate	2.67	1.45	0.97	0.48	2.40	1.69	1.61	0.08	1.51	2.06	2.01	0.05
1169	Ammoniated superphosphate	13.05	1.63	1.44	0.19	12.40	2.06	1.70	0.36	12.24	2.42	2.23	0.19
1518	Ammoniated double superphosphate	28.40	1.30	1.07	0.23	27.23	2.43	2.20	0.23	27.50	4.09	3.50	0.59
1508	Mixed fertilizer	3.35	0.43	0.32	0.11	3.07	0.68	0.60	0.08	3.01	1.46	1.17	0.29
1522	Mixed fertilizer	10.54	0.12	0.02	0.10	10.00	0.38	0.46	−0.08	9.82	1.36	1.75	−0.39
1524	Mixed fertilizer	5.65	0.17	0.08	0.09	5.44	0.44	0.20	0.24	5.50	1.16	0.79	0.37

[a] Washed on a 9 cm. paper by gravity filtration to a total volume of 225 ml.; approximately 11 ml. of cold water was used at each washing.

[b] Insoluble P_2O_5 in unwashed sample minus insoluble P_2O_5 in washed sample.

washed with successive small portions of cold water prior to the citrate digestion, usually showed small but progressive decreases as the total volumes of wash water were increased by 50 ml. increments from 100 ml. to 250 ml. Although they are not entirely consistent, the results given in Table 5 indicate that with 1 gram charges, washed on a 9 cm. paper with successive 11 ml. portions of cold water, the figures for citrate-insoluble phosphorus in ordinary and double superphosphates are affected little by increasing the total volume of wash water beyond 75 ml. However, with ammoniated ordinary superphosphate, heavily limed superphosphate, and wet-mixed base goods important decreases in the percentages of citrate-insoluble phosphorus were usually obtained by using larger total volumes of wash water. Although by far the greater portion of the water-soluble phosphorus was removed in the first 75 ml. of water, further progressive increases in the percentages of water-soluble phosphorus were invariably obtained by increasing the total volume of wash water.

TABLE 5.—*Effect of amount of washing, prior to citrate digestion, on results for citrate-insoluble phosphorus*

(Analyses made on 1 gram charges. Results expressed in percentage.)

SAMPLE	MATERIAL	CITRATE-INSOLUBLE P_2O_5				WATER-SOLUBLE P_2O_5		
		VOLUME OF WASH WATER[a]				VOLUME OF WASH WATER[a]		
		UN-WASHED	75 ML.	150 ML.	225 ML.	75 ML.	150 ML.	225 ML.
1370	Superphosphate	0.73	0.53	0.50	0.57	18.05	18.25	18.32
1378	Superphosphate	4.34	3.95	3.88	3.83	11 70	11.98	12.63
1500	Superphosphate	0.22	0.10	0.15	0.14	14.50	14.58	14.60
1509	Superphosphate	0.22	0.15	0.11	0.07	14 05	14.55	14.68
1337	Double superphosphate	0.49	0.12	0.09	0.19	37.93	38.28	38.83
1368	Double superphosphate	3.11	2.83	2.91	2.89	39.08	40.45	41.05
1481	Double superphosphate	0.81	0.64	0.55	0.57	27.55	29.20	31.20
1168	Ammoniated superphosphate	8.61	7.85	7.73	7.64	0.10	0.24	0.32
1175	Ammoniated superphosphate	2.55	2.04	1.63	1.49	7.21	7.49	7.63
1177	Ammoniated superphosphate	3.22	2.87	2.72	2.60	8.83	9.25	9.85
1185	Limed superphosphate	8.76	7.51	6.20	5.42	0.43	0.60	0.69
1105	Wet-mixed base goods	3.36	3.27	3.19	2.98	5.00	5.10	5.41
1525	Mixed fertilizer	0.73	0.28	0.14	0.19	6.80	6.85	7.00
1526	Mixed fertilizer	0.25	0.17	0.14	0.12	7.60	7.75	7.95

[a] Washed on a 9 cm. paper by gravity filtration, approximately 11 ml. of cold water was used at each washing.

The effects of varying the quantity of water used at each washing and of using different sizes and grades of filter paper were not studied in the work described in this paper. The few data reported by Austin (*1*) indi-

cate, however, that practically the same results for citrate-insoluble phosphorus are obtained by washing a 2 gram charge 10 times on a 11 cm. paper, using 25 ml. each time, as by washing the charge on a 9 cm. paper to the same total volume. The data reported by MacIntire, Jones and Hardin (*12*) indicate that practically the same results for water-soluble phosphorus and citrate-insoluble phosphorus in double superphosphate and other calcium and magnesium phosphates that are high in water-soluble phosphorus are obtained whether the water-soluble phosphorus is removed by washing the charge on a paper by gravity filtration or by washing on a Shimer filter by suction.

SOLUBILITY OF THE PRINCIPAL SUPERPHOSPHATE CONSTITUENTS IN WATER AND IN NEUTRAL AMMONIUM CITRATE SOLUTION

Data reported by Jacob, Hill, Ross and Rader (*5*) show that in addition to the phosphorus the greater portions of the calcium, iron, aluminum, and fluorine and all or nearly all the sulfur in ordinary and double superphosphates are dissolved by the water and citrate extractions during the determination of citrate-insoluble phosphorus. Further data, obtained by Hill (*3*) on ordinary and double superphosphates that contained no added filler or conditioner, are given in Table 6. Inasmuch as these results were obtained on 2 gram charges and the citrate digestions were carried on for only 30 minutes, they are probably somewhat lower than would be obtained with the 1 gram charge in accordance with the present official method.

In the case of ordinary superphosphate the water extraction dissolves not only the greater portion of the total phosphorus but also a considerable portion of the total sulfate, which is present principally in the form of anhydrite (*4*), and all or nearly all the remaining sulfate is dissolved by the ammonium citrate solution. Monocalcium phosphate is the principal water-soluble phosphatic constituent of ordinary and double superphosphates. The nature of the citrate-soluble phosphorus in ordinary and double superphosphates that contain no added filler or conditioner is not definitely known, but there is strong evidence that in many samples it consists, at least in part, of iron phosphate and/or aluminum phosphate (*7*).

The compounds present in ammoniated ordinary superphosphate depend to some extent upon the amount of ammonia added (*5, 9*). Thus, with different degrees of ammoniation the possible principal components are mono-, di-, and tricalcium phosphate, monoammonium phosphate, calcium sulfate, and ammonium sulfate. Also depending upon the amount of ammonia added, the possible principal components of ammoniated double superphosphate are mono-, di-, and tricalcium phosphates and mono- and diammonium phosphates (*18*). Water-insoluble phosphates are formed when either ground limestone or dolomite is mixed with super-

TABLE 6.—*Solubilities of the principal superphosphate constituents in water and in neutral ammonium citrate solution*

(2 gram charge washed with cold water on a 9 cm. paper to a total volume of approximately 225 ml., and water-insoluble residue digested with 100 ml. of neutral ammonium citrate solution for 30 minutes at 65°C. Results expressed in percentage.)

MATERIAL	P_2O_5			F			SO_3[a]		
	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE
Ordinary superphosphate[b]	19.20	16.70	2.38	1.56	0.25	0.92	29.50	12.66	16.86
Ordinary superphosphate[b]	20.22	17.01	3.16	1.65	0.29	1.29	29.29	9.33	19.16
Ordinary superphosphate[c]	18.86	12.30	6.29	1.49	0.23	1.11	30.44	12.51	17.33
Ordinary superphosphate[c]	19.88	13.63	5.89	1.30	0.28	0.78	30.60	9.75	20.90
Double superphosphate[b,d]	46.21	38.30	7.42	2.04	0.47	1.26	3.19	2.85	0.00
Double superphosphate[b,d]	49.27	41.63	7.57	1.85	0.57	0.95	1.90	1.94	0.00
Double superphosphate[c,e]	48.37	42.20	5.69	1.15	0.31	0.49	2.10	1.65	0.46
Double superphosphate[f,d]	47.33	41.30	3.66	1.81	0.88	0.47	2.74	2.69	0.00

MATERIAL	CaO			Al_2O_3			Fe_2O_3		
	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE	TOTAL	WATER-SOLUBLE	CITRATE-SOLUBLE
Ordinary superphosphate[b]	27.20	14.21	12.70	0.56	0.12	0.41	0.80	0.02	0.80
Ordinary superphosphate[b]	28.10	13.06	15.00	0.64	0.00	0.67	0.64	0.06	0.60
Ordinary superphosphate[c]	26.29	12.09	14.20	1.33	0.00	1.18	1.80	0.25	1.46
Ordinary superphosphate[c]	27.59	11.86	15.73	0.84	0.00	0.86	2.06	0.08	1.72
Double superphosphate[b,d]	18.62	15.85	2.71	1.40	0.04	1.38	2.04	0.13	1.63
Double superphosphate[b,d]	18.00	15.91	2.09	1.27	0.06	1.23	1.62	0.13	1.52
Double superphosphate[c,e]	18.48	16.80	1.63	0.98	0.06	0.76	1.25	0.14	1.00
Double superphosphate[f,d]	19.64	15.30	1.10	1.73	0.85	0.62	0.83	0.08	0.64

[a] Sulfates only.
[b] Commercial material prepared from Florida pebble phosphate.
[c] Commercial material prepared from Tennessee brown-rock phosphate.
[d] Phosphoric acid was made by the H_2SO_4 process.
[e] Phosphoric acid was made by the blast furnace process.
[f] Made commercially from Idaho phosphate

phosphates (*11, 13, 14*) or with ammonium phosphates (*2*). Obviously a wide variety of compounds may be present in complete fertilizer mixtures.

It has been shown (*6*) that calcium sulfate, monocalcium phosphate, calcium carbonate, calcium fluoride, magnesium sulfate, magnesium carbonate, dolomite, diammonium phosphate, and precipitated aluminum and ferric oxides tend to decrease the solubilities of di- and tricalcium phosphates in neutral ammonium citrate solution, whereas ammonium sulfate, sodium nitrate, potassium sulfate, potassium chloride, and monoammonium phosphate tend to increase the solubilities of these phosphates. Under comparable conditions, the effects of the compounds of the first group in decreasing the solubilities of di- and tricalcium phosphates are usually somewhat greater than those of the second group in increasing the solubilities. No information is available concerning the effects of various compounds on the citrate solubilities of iron and aluminum phosphates.

These data indicate that, as compared with the results obtained on unwashed charges, removal of the water-soluble compounds prior to the citrate digestion may result in either higher or lower figures for citrate-insoluble phosphorus, depending on the nature and quantity of the water-soluble compounds and of the water-insoluble phosphates in the sample. However, the results given in the present paper show that the figures obtained on unwashed charges are usually higher than those on washed charges. In the few exceptions observed in this study (Tables 2 and 3) the lower figures obtained on the unwashed charges cannot be explained on the basis of the present knowledge of composition of the samples. Furthermore, the determinations on the 1 gram charges of the ammoniated double superphosphate No. 1520 (Table 2) and the 2 gram charges of the mixed fertilizer No. 1522 (Table 3) were repeated several times and the differences were always in the same direction.

SUMMARY

A study was made of the effect of removing water-soluble compounds prior to the determination of citrate-insoluble phosphorus in 43 samples of ordinary superphosphates, double superphosphates, ammoniated ordinary superphosphates, ammoniated double superphosphates, wet-mixed base goods, and complete fertilizer mixtures.

With very few exceptions the results for citrate-insoluble phosphorus are lower on washed charges than on the corresponding unwashed charges. In general, the smallest differences between results on washed and unwashed charges are obtained with ordinary and double superphosphates and the largest with ammoniated ordinary superphosphates and heavily limed superphosphates. Varying the weight of the sample between the limits 0.5 gram and 2 grams usually does not have a uniform effect on the differences between the results obtained on washed and unwashed charges.

With ordinary and double superphosphates it makes little difference in the results for citrate-insoluble phosphorus whether the charge is washed with 75 ml. or 225 ml. of water prior to the citrate digestion. On the other hand, thorough washing of the sample as directed in the official method seems to be important in the case of ammoniated ordinary superphosphate, heavily limed superphosphate, and wet-mixed base goods.

Data are given on the solubilities of the principal superphosphate constituents in water and in neutral ammonium citrate solution. The effects of various compounds on the solubilities of phosphates in citrate solution are discussed.

LITERATURE CITED

(1) Austin, W. R. *Ind. Eng. Chem.*, **15**, 1037–8 (1923).
(2) Beeson and Ross. *Ibid.*, **26**, 992–7 (1934).
(3) Hill, W. L. Unpublished data.
(4) Hill and Hendricks. *Ind. Eng. Chem.*, **28**, 440–7 (1936).
(5) Jacob, Hill, Ross and Rader. *Ibid.*, **22**, 1385–92 (1930).
(6) Jacob, Rader, Marshall, and Beeson. *Ind. Eng. Chem. Anal. Ed.*, **4**, 25–31 (1932).
(7) Jacob, Rader and Ross. *J. Assoc. Official Agr. Chem.*, **15**, 146–62 (1932).
(8) Jacob, Rader and Tremearne. *Ibid.*, **19**, 449–72 (1936).
(9) Keenen, F. G. *Ind. Eng. Chem.*, **22**, 1378–82 (1930).
(10) Lapp, M. E. *J. Assoc. Official Agr. Chem.*, **15**, 65–87 (1932).
(11) MacIntire, Hardin and Oldham. *Ind. Eng. Chem.*, **28**, 711–17 (1936).
(12) MacIntire, Jones and Hardin. *J. Assoc. Official Agr. Chem.*, **18**, 301–6 (1935).
(13) MacIntire and Shaw. *Ind. Eng. Chem.*, **24**, 1401–9 (1932).
(14) MacIntire and Shuey. *Ibid.*, **24**, 933–41 (1932).
(15) *Methods of Analysis, A.O.A.C.*, 4th ed., 1935.
(16) Ross and Jacob. *J. Assoc. Official Agr. Chem.*, **14**, 182–96 (1931).
(17) Ross, Jacob and Beeson. *Ibid.*, **15**, 227–66 (1932).
(18) White, Hardesty and Ross. *Ind. Eng. Chem.*, **27**, 562–7 (1935).

FURTHER STUDIES ON THE DETERMINATION OF AVAILABLE POTASH IN FERTILIZERS*

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In studies previously reported it was shown by the writers[1] and by Ross, Beeson, White, and Merz[2] that extraction with boiling water, as required in the former official method, often fails to remove all available

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[1] *This Journal*, **18**, 260, 281 (1935); Commercial Fertilizer Year Book, 33–38 (1935).
[2] *This Journal*, **18**, 327 (1935).

potash from fertilizer materials. Boiling with dilute salt solutions was shown to give much more complete removal of the available potash.

As a result of these studies Kraybill[1] recommended to the Association of Official Agricultural Chemists that boiling with a dilute solution of ammonium oxalate be substituted for extraction with boiling distilled water. This recommendation was approved, and the procedure was adopted as official at the 1935 meeting of the Association.

Additional data obtained in recent work with several somewhat unusual fertilizers are reported in this paper. These data, it is hoped, will be of assistance in promoting a fuller understanding and more satisfactory use of the present official method.

One fertilizer from the 1933 Indiana inspection, sample HH-9733, Table 1, was found to give an abnormally large increase for the present as compared to the former official method. As was to be expected, residues resulting from the extraction of this material with boiling distilled water contained unusually large amounts of available potash.

TABLE 1.—*Comparison of present and former official methods for determining available potash in fertilizers*

SAMPLE NO.	ANALYSIS	AVAILABLE K_2O IN FERTILIZER			AVAILABLE K_2O IN EXTRACTED RESIDUE	
		FORMER METHOD	PRESENT METHOD	INCREASE FOR PRESENT METHOD	NEUBAUER METHOD	AMMONIUM OXALATE EXTRACTION
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
HH-9733	3–18–9	9.26	11.92	1.66	0.98	2.18
K-58	"Flue Dust"	5.19	15.55	10.36	9.7	—

Investigation of the materials used in the manufacture of this fertilizer indicated that "flue dust," a by-product obtained in the manufacture of phosphoric acid by the volatilization process was responsible for the unusual results obtained. This material contains a relatively high percentage of available phosphorus and considerable silica. Comparative data for available potash by the present and former official methods together with the Neubauer value for available potash in the residue after extraction with boiling distilled water are given in Table 1, Sample K-58.

The available potash values obtained with the present official method are much higher than those obtained with the former method, 15.55 and 5.19 per cent potassium oxide respectively. The Neubauer value for available potash in the extracted residue indicates that the 10.36 per cent potassium oxide increase with the present method represents potash actually available to plants. This is confirmed by data obtained in pot tests with barley on an acid Crosby silt loam soil (Fig. 1). The responses

[1] *This Journal*, **18**, 237 (1935); **19**, 302 (1936).

indicated in Table 2 are taken as a measure of the available potash content of the extracted residue or of the fertilizer added.

FIG. 1.—POT TESTS SHOWING POTASH RESPONSE TO RESIDUE FROM SAMPLE OF "FLUE DUST" EXTRACTED ACCORDING TO THE FORMER OFFICIAL METHOD FOR DETERMINING AVAILABLE POTASH IN FERTILIZERS.
201—Check (NP only).
203— 5 grams residue.
205—10 grams residue.
207—5 grams "flue dust" (not extracted).

For 5 grams of the extracted residue a four-fold increase in dry weight is indicated. Ten grams of residue gave a slightly higher response. Only

TABLE 2.—*Available potash content of "flue dust" residue after extraction with boiling distilled water as shown by pot tests*

FERTILIZER	TOTAL DRY WEIGHT	INCREASE OVER CHECK	
		DRY WEIGHT	DRY WEIGHT
	grams	*grams*	*per cent*
Check (NP only)	13	—	—
5 grams Extracted Residue	65	52	400
10 grams Extracted Residue	77	64	492
5 grams Fertilizer (Not Extracted)	69	56	430

slightly lower response was obtained with 5 grams of extracted residue than with 5 grams of the original fertilizer, indicating that the extracted residue contained almost as much available potash as the unextracted material. This indication is confirmed by data given in Table 3.

TABLE 3.—*Effect of extraction with boiling distilled water on the available potash content of "flue dust"*

FERTILIZER EXTRACTED	RESIDUE OBTAINED		K_2O EXTRACTED		AVAILABLE POTASH BY PRESENT OFFICIAL METHOD			
					ORIGINAL FERTILIZER		EXTRACTED RESIDUE	
grams	*grams*	*per cent*	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*grams*
60.0	48.0	80.0	5.19	3.11	15.55	9.33	13.00	6.24

With some fertilizers, the present official method gives water-insoluble residues in the potassium chloroplatinate precipitate. If not eliminated through the prescribed use of acidulated alcohol, these residues may be sufficiently large to cause appreciable errors when determinations are made by direct weighings. This was reported in a previous paper (3) but no data were presented regarding the amount and composition of the residues.

TABLE 4.—*Composition of insoluble residue contained in potassium chloroplatinate precipitate*

SAMPLE NO.	ANALYSIS	LOSS ON IGNITION	IGNITED RESIDUE	COMPOSITION OF IGNITED RESIDUE				
				SiO_2	$AlPO_4$	$FePO_4$	$Mg_3(PO_4)_2$	UNDETERMINED
		per cent	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
HH-9733	3-18-9	25.3	186.7	31.0	93.0	28.3	5.3	29.1
K-56	2-9-5	21.9	195.3	0.5	105.7	28.3	5.8	55.0
K-57	2-12-6	29.4	176.5	4.0	100.3	24.7	6.2	41.3

From the data given in Table 4 it will be seen that the insoluble residues are made up largely of iron and aluminum phosphates, with aluminum phosphate present in by far the larger amounts. Silica and calcium and magnesium phosphates are present in only minor quantities. Apparently, in the presence of the comparatively large amount of ammonium oxalate, there is an incomplete removal of iron and aluminum by precipitation with ammonium hydroxide. On the other hand calcium appears to be completely eliminated.

Some data relative to the amounts of insoluble residue found and the effectiveness of the use of acidulated alcohol in eliminating this insoluble material are given in Table 5. The acidulated alcohol was produced by adding 0.6 cc. of concentrated hydrochloric acid to the alcohol with which the potassium platinic chloride precipitate was taken up in the platinum dish prior to filtration; this solution was rubbed up well with a rubber policeman and allowed to stand 10–15 minutes before being filtered.

The data presented show that with a number of fertilizers no appreciable insoluble residues were obtained with any method of extraction used. However, in general, the present official method without acidulated alcohol gave considerably higher insoluble residues than did the former

method. In a few cases such values were quite high. In contrast no very high values were obtained with the former method, whether or not hydrochloric acid was used.

TABLE 5.—*Effectiveness of acidulated alcohol in eliminating insoluble residue accompanying potassium chloroplatinate precipitate*

SAMPLE NO.	ANALYSIS	INSOLUBLE RESIDUE AS PER CENT K_2O					
		FORMER METHOD		PRESENT METHOD		INCREASE FOR PRESENT METHOD	
		WITHOUT HCl	WITH HCl	WITHOUT HCl	WITH HCl	WITHOUT HCl	WITH HCl
HH-9733	3-18-9	0.00	0.00	0.42	0.00	0.42	0.00
HH-9753	3-18-9	0.00	0.00	0.34	0.03	0.34	0.03
HH-9784	3-8-6	0.00	0.00	0.10	0.00	0.10	0.00
HH-9792	0-12-12	0.07	0.05	0.04	0.00	−0.03	−0.07
HH-9807	5-10-5	0.05	0.04	0.01	0.00	−0.04	−0.05
HH-9815	2-12-2	0.02	0.02	0.13	0.00	0.11	−0.02
HH-9819	0-14-7	0.09	0.06	0.09	0.05	0.00	−0.04
HH-9838	1-8-4	0.05	0.02	0.24	0.05	0.19	0.00
HH-9841	0-14-6	0.06	0.03	0.23	0.06	0.17	0.00
HH-9884	0-12-12	0.01	0.00	0.00	0.00	−0.01	−0.01
HH-9889	2-12-2	0.00	0.00	0.11	0.00	0.11	0.00
HH-9916	0-10-10	0.01	0.00	0.00	0.00	−0.01	−0.01
HH-9917	3-15-12	0.03	0.07	0.24	0.01	0.21	−0.02
HH-9918	2-12-6	0.04	0.02	0.13	0.01	0.09	−0.03
HH-9919	3-9-18	0.13	0.08	0.10	0.08	−0.03	−0.05
HH-9929	0-12-12	0.00	0.00	0.00	0.00	0.00	0.00
HH-9933	2-12-6	0.00	0.00	0.06	0.02	0.06	0.02
HH-9942	2-12-6	0.00	0.00	0.06	0.01	0.06	0.01
HH-9943	2-12-6	0.02	0.00	0.23	0.01	0.21	−0.01
HH-9950	2-12-2	0.02	0.01	0.03	0.03	0.01	0.01
HH-9963	0-10-10	0.05	0.00	0.06	0.01	0.01	−0.04
HH-9969	2-10-4	0.02	0.00	0.19	0.04	0.17	0.02
HH-9973	0-12-12	0.00	0.06	0.09	0.09	0.09	0.09
HH-9984	4-8-7	0.05	0.02	0.14	0.03	0.09	−0.02
K-56	2-9-5	0.01	0.01	0.24	0.08	0.23	0.07
K-57	2-12-6	0.03	0.00	0.20	0.10	0.17	0.07
K-58	"Flue Dust"	0.10	0.10	0.24	0.10	0.14	0.00
Average		0.03	0.02	0.14	0.03	0.10	0.00
Maximum		0.13	0.10	0.42	0.10	0.42	0.09
Minimum		0.00	0.00	0.00	0.00	−0.04	−0 07

The use of acidulated alcohol in the present official procedure almost entirely eliminated the insoluble residue. In this case the possible error in the present official method is no greater than in the former procedure. Thus the maximum values for the former procedure and the present method both with and without the use of acidulated alcohol are 0.13,

0.10, and 0.42 per cent potassium oxide, respectively. The corresponding average values are 0.03, 0.03, and 0.14 per cent potassium oxide, respectively.

SUMMARY AND CONCLUSIONS

As an extension of studies already reported, data are presented for a number of somewhat unusual fertilizer materials.

With one such material ("flue dust") available potash values are 5.19 per cent potassium oxide by the former official method and 15.55 per cent potassium oxide by the present official method, a difference of 10.36 per cent. Data obtained with both the Neubauer method and pot tests indicate that the higher value obtained with the present method represents the potash actually available to plants.

With some fertilizers, the use of the present official method gave water-insoluble residues in the potassium chloroplatinate precipitate. If not eliminated through the prescribed use of acidulated alcohol, these residues may be sufficiently large to cause appreciable errors when determinations are made by direct weighings. However, for the majority of materials, such residues were quite small.

Analyses of several of these residues showed them to be composed principally of iron and aluminum phosphates with aluminum phosphate present in by far the larger amount. Calcium and magnesium compounds were present in only minor quantities.

The addition of 0.6 cc. of concentrated hydrochloric acid to the alcohol with which the potassium chloroplatinate precipitate is taken up in the platinum dish before filtration, as prescribed in the present official method, eliminated the insoluble residues with all fertilizers studied. The use of this acidulated alcohol led to practically identical results regardless of whether the determination was made by direct weighing or by difference after leaching. Insoluble residues obtained with this procedure were no larger than those obtained with the former official method.

ACKNOWLEDGMENT

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THE SIMULTANEOUS QUANTITATIVE ESTIMATION OF THE PERCENTAGES METHOXYL AND ETHOXYL GROUPS IN ORGANIC SUBSTANCES

By MAX PHILLIPS and M. J. GOSS*

The method for the quantitative estimation of the percentage of methoxyl ($-OCH_3$) in an organic substance, first developed by Zeisel,[1] is based

* Contribution No. 277 from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, U.S. Department of Agriculture, Washington, D. C.

[1] *Monatsh.*, 6, 989 (1885); 7, 406 (1886).

on a reaction that all ethers undergo when heated with hydriodic acid. The reaction may be represented as follows:

$ROR'+HI=ROH+R'I$, in which R may be either an alkyl or an aryl group, and R' an alkyl group. If R' is a methyl group, that is, if the substance contains a methoxyl group, then methyl iodide is formed as a result of the reaction. The methyl iodide produced may be absorbed in a suitable medium, such as an alcoholic solution of silver nitrate, pyridine or a solution of bromine and potassium acetate in glacial acetic acid, and the iodine is determined either gravimetrically or volumetrically. A review of the literature on the determination of the percentage of methoxyl was presented in a previous paper.[1]

The percentage of ethoxyl ($-OC_2H_5$) in an organic substance may be determined by the same general method, but when a substance contains both the methoxyl and ethoxyl groups, it is not possible to determine the percentages of these groups by the Zeisel method, as then a mixture of methyl and ethyl iodides is obtained.

Friedrich[2] has proposed a micro method for the determination of the percentages methoxyl and ethoxyl, when both of these groups are present in the same substance. He first determines the combined weight of silver iodide afforded by the substance when analyzed by the micro-Zeisel method. In a second sample, the mixture of methyl and ethyl iodides yielded when the substance is heated with hydriodic acid is passed through a combustion tube, and the percentage carbon is determined. From the results of the two determinations, the percentages of methoxyl and ethoxyl are calculated. Aside from the fact that two separate determinations are required in the Friedrich method, there is the objection to the combustion procedure, which, at best, is a troublesome operation.

Willstätter and Utzinger,[3] in connection with their work on chlorophyll made a separation of methyl and ethyl iodides by combining them with trimethylamine, obtaining a mixture of tetramethylammonium iodide and trimethylethylammonium iodide, and then separating these salts by means of absolute ethanol. Wilson[4] found that at 25°C., 100 cc. of absolute ethanol dissolves 0.040 gram of the tetramethylammonium iodide, and at the same temperature 4.165 grams of the trimethylethylammonium iodide dissolves in 100 cc. of absolute ethanol.

The method described in this paper is based on the Willstätter-Utzinger principle. After the tetramethylammonium iodide has been separated from the trimethylethylammonium iodide with abolute ethanol, each is precipitated with an aqueous silver nitrate solution and weighed as silver iodide. A correction is made for the quantity of tetramethylammonium iodide dissolved in the ethanol under the experimental conditions specified.

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[1] *This Journal*, **15**, 118 (1932).
[2] *Microchem.*, **7**, 185 (1929).
[3] *Ann. Chem.*, **382**, 148 (1911).
[4] *This Journal*, **18**, 477 (1935).

The modified method follows:

APPARATUS

The apparatus is a modified form of the Zeisel-Fanto[1] apparatus for the determination of the percentage methoxyl. It consists of a reaction flask, C, which is immersed in a glycerol bath, Q. C is connected by means of a No. 15 interchangeable ground-glass joint to the inner tube of condenser R, which in turn is connected through another interchangeable ground-glass joint (No. 7) to the scrubbing flask D. This flask is filled to approximately one-fourth its capacity with a thin suspension of purified* red phosphorus in distilled water for the purpose of freeing the alkyl iodides of any iodine vapor that may come over. D is connected by means of an interchangeable No. 7 ground-glass joint to U-tube G, which is filled as follows: A small wad of cotton is placed in the top of the constricted end of this tube and it is then half-filled with anhydrous $CaCl_2$ as indicated by the line near F. The portion of the tube from F to the lower limit of E (indicated by a line in the drawing) is filled with a mixture of short fibers of glass wool and anhydrous $CaCl_2$. The portion of the tube marked E is filled with glass wool, and the ground-glass joint is greased with stopcock grease. D and U-tube G are immersed in a 1000 cc. beaker, P, which is filled with water maintained at 70°C. G is connected through a No. 7 interchangeable ground-glass joint to the tube H, which in turn is connected to the absorption flasks J, K and L, and flask M; J, K, and L are also connected by means of No. 7 interchangeable ground-glass joints. M contains dilute sulfuric acid to prevent the trimethylamine from escaping into the air. All ground-glass joints are held fast by means of bronze springs, N, attached to glass hooks. Water from the 500 cc. Erlenmeyer flask B, heated to 70°C., is circulated through the condenser R. The water overflowing from R is caught in the Erlenmeyer flask O. A stream of carbon dioxide from a tank provided with a reducing valve is passed through the sulfuric acid wash bottle A into the apparatus.

PROCEDURE

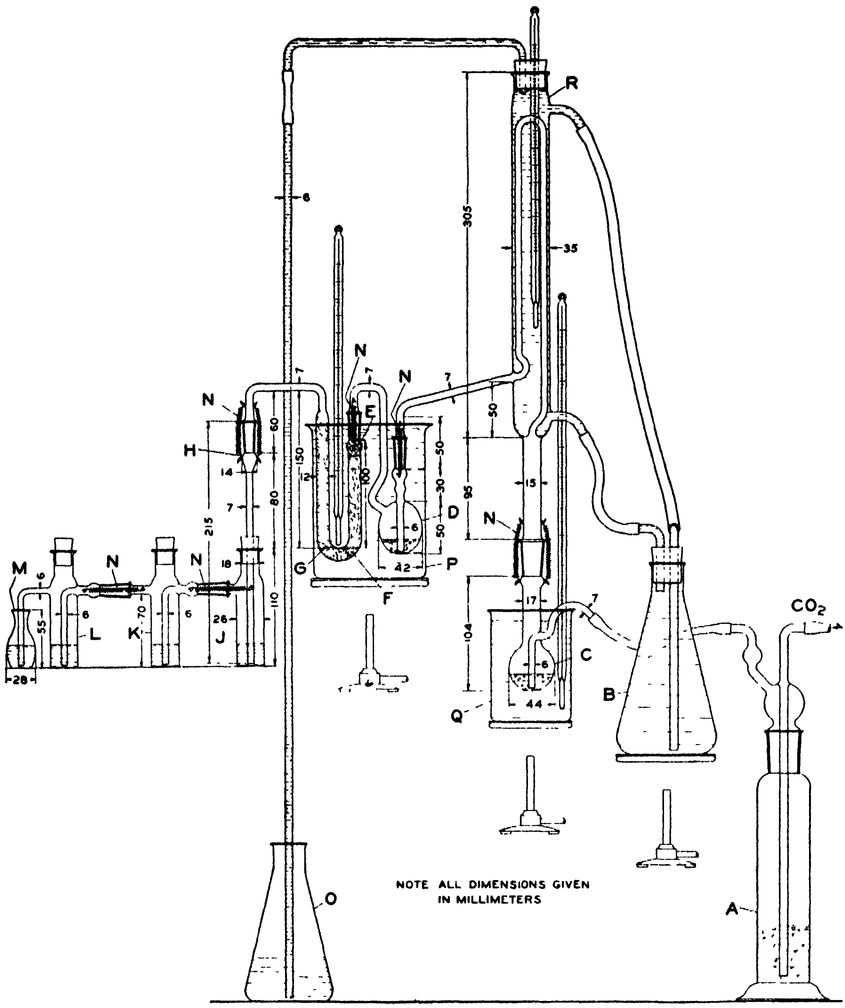
The temperature of the water of condenser R and bath P is raised to 70°C. and maintained at that temperature throughout the determination. To each of the absorption flasks (J, K, and L) are added 5 cc. of a 10% solution (by weight) of anhydrous trimethylamine† in absolute ethanol (99.8% ethanol by weight) and 10 cc. of absolute ethanol. Dilute sulfuric acid is added to flask M until it is approximately half-full. The ground-glass joints between J, K, and L, and between G and H are wetted with a few drops of absolute ethanol. The absorption flasks and tube H are then connected, and bronze springs are attached. The weighed sample (0.1–0.3 g.) is placed in reaction flask C, and 10 cc. of hydriodic acid (sp. gr. 1.70), 3 cc. of phenol, and a piece of porous tile are added. The ground-glass joint between C and R is wetted with a few drops of hydriodic acid, and the flask C is connected to the apparatus and held fast by means of bronze springs, N. A stream of carbon dioxide gas is led through the apparatus at the rate of 55–60 bubbles per minute. The reaction flask is inserted in the glycerol bath Q, which is heated to 130–140°C., and the heating is continued for 1½ hours. H is then disconnected from G, and M is removed from L. J, K, and L are disconnected, the top of H and the openings of the absorption flasks are stoppered, and the flasks are allowed to stand at room temperature for 24 hours. The contents of absorption flasks K and L are transferred to a 400 cc. beaker (I), K and L are washed with absolute ethanol, and the alcoholic wash-

[1] *Z. Anal. Chem.*, 42, 554 (1903).

* Purified by digesting with dilute ammonia on the water bath for 30 min., filtering, and washing with distilled water. The purified red phosphorus is preserved in the wet state in a well stoppered bottle.

† Purchased from Eastman.

ings are also added to I. The beaker I is placed on the steam bath, and the alcoholic solution is allowed to evaporate to dryness. The tube H is removed from J with care to avoid loss of any of the crystalline substance adhering to it and washed with 5 cc. of absolute ethanol, the washings are filtered through a small filter paper (6–7



APPARATUS FOR DETERMINATION OF PERCENT METHOXYL AND ETHOXYL

cm. in diameter) into a 100 cc. beaker (II). The clear supernatant liquid in J is filtered through the same filter paper, and the filtrate is also collected in beaker II. To the crystalline tetramethyl-ammonium iodide, 5 cc. of absolute ethanol is added, the mixture is stirred with a glass rod, the crystals are allowed to settle, and the clear alcoholic solution is filtered through the filter paper into beaker II. This oper-

ation is repeated with four other 5 cc. portions of absolute ethanol. The content of beaker II is then evaporated to dryness on the steam bath, and the beaker and residue are allowed to cool in a desiccator. To beaker II, 20 cc. of absolute ethanol is then added, and the mixture is stirred well with a glass rod and filtered through the filter paper and funnel previously used. The filtrate from this operation is collected in beaker I. Beaker II is washed successively with five 5 cc. portions of absolute ethanol, and the alcoholic washings are passed through the same filter paper and funnel into beaker I. To the filtrate in beaker I are also added 250 cc. of distilled water, 10 drops of concentrated HNO_3, and an excess of a 5% aqueous $AgNO_3$ solution. This mixture is digested on the steam bath for 2 hours and then allowed to cool to room temperature. The crystals of tetramethyl-ammonium iodide in J are dissolved in a few cc. of distilled water, and the solution is transferred to a 400 cc. beaker (III). J is thoroughly washed with distilled water, and the washings are added to beaker III. Similarly, the tetramethylammonium iodide adhering to H and that on the filter paper and in beaker II are washed into beaker III by means of a stream of distilled water. The solution in beaker III is diluted with distilled water until the volume is approximately 300 cc., 10 drops of concentrated HNO_3 and an excess of a 5% aqueous $AgNO_3$ solution are added, and the mixture is heated on the steam bath for 2 hours and then allowed to cool to room temperature. The silver iodide precipitates in beakers I and III are filtered separately into weighed Gooch crucibles, dried at 105°C., and weighed. To the weight of AgI obtained from beaker III, 0.0110 gram is added, as this corresponds to the weight of AgI yielded by the tetramethylammonium iodide that dissolves in the absolute ethanol under the experimental conditions specified. Similarly, from the weight of AgI obtained from beaker I, 0.0110 gram is deducted. The percentages of methoxyl and ethoxyl are calculated as follows:

$$\frac{\text{Wt. of AgI from beaker III}+0.0110\times0.1321\times100}{\text{Wt. of sample}}=\text{per cent }-OCH_3;$$

$$\frac{\text{Wt. of AgI from beaker I}-0.0110\times0.1918\times100}{\text{Wt. of sample}}=\text{per cent}-OC_2H_5.$$

A few typical results obtained with several mixtures of pure compounds containing the methoxyl and ethoxyl groups are given in Table 1.

TABLE 1.—*Percentages of methoxyl and ethoxyl*

SUBSTANCES TESTED		WEIGHT OF AgI FROM BEAKER III[1]	WEIGHT OF AgI FROM BEAKER I[2]	FOUND		CALCULATED	
ANISIC ACID	P-ETHOXY BENZOIC ACID			$-OCH_3$	$-OC_2H_5$	$-OCH_3$	$-OC_2H_5$
gram	*gram*	*gram*	*gram*	*per cent*	*per cent*	*per cent*	*per cent*
0.2000	0.1000	0.3069	0.1422	13.51	9.09	13.59	9.03
0.2000	0.1000	0.3073	0.1386	13.53	8.86	13.59	9.03
0.1500	0.1500	0.2204	0.2101	9.70	13.43	10.19	13.55
0.1000	0.1000	0.1510	0.1380	9.97	13.23	10.19	13.55
0.1500	0.1000	0.2259	0.1388	11.93	10.65	12.23	10.84
0.2000	0.1000[3]	0.2969	0.1423	13.07	9.10	13.59	9.03

[1] Weight of AgI actually obtained plus 0.0110 g.
[2] Weight of AgI actually obtained minus 0 0110 g.
[3] 3, ethoxy-4-hydroxy-benzaldehyde was used instead of p-ethoxy benzoic acid.

SUMMARY

A method is described for the determination of the percentages methoxyl and ethoxyl when both of these groups are present in the same compound or in mixtures of compounds.

FURTHER STUDIES OF FLUORINE DISTILLATION

By Dan Dahle and H. J. Wichmann
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Washington, D. C.)

The influence of certain factors on the recovery of fluorine in a distillation with sulfuric acid has been described by the writers.[1] The work has now been extended to include distillations with perchloric acid and phosphoric acid, since both these acids are being used in procedures for fluorine volatilization. The effect of the presence of salts of nonvolatile acids was also studied.

1. EFFECT OF INPUT VOLUME

By "input volume" is meant the volume present in the distilling flask when the temperature at which the distillation is to be made is reached. The relationship between input volume and temperature for perchloric acid (60% strength) and for phosphoric acid (85% strength) was found. It is given in Table 1.

Table 1.—*Boiling point of mixtures of H_2O with $HClO_4$ and H_3PO_4*

TEMP.	$HClO_4$ (60%)	H_2O	INPUT VOLUME
°C.	cc.	cc.	cc.
125	10	4.5	14.5
135	10	2.5	12.5
142	10	0.5	10.5
	H_3PO_4 (85%)		
125	10	4	14
135	10	2	12

Next, mixtures of perchloric acid giving different input volumes, but containing the same amount of fluorine as NaF, were distilled at constant temperature to a constant output volume. The input volume was varied in two ways: (1) By increasing the amount of perchloric acid and (2) by adding salts of non-volatile acids. The salts used were sodium perchlorate and calcium monophosphate. The conditions of the experiment were:

Temperature: Constant at either 125° or 135°C.
Output volume: Constant at 25 cc.
Fluorine as NaF: Constant at 0.5 mg.
Input volume: Varying.

* W. B. White, Chief.
[1] *This Journal*, 19, 313 (1936).

The results are shown in Table 2.

TABLE 2.—*Effect of input volume on recovery*

TEMP.	$HClO_4$	SALT	INPUT VOLUME	RECOVERY	CHANGE IN RECOVERY PER CC. I.V.
°C.	cc.	grams	cc.	per cent	per cent
125	10	none	14.5	81.6	1.062
125	20	none	29.0	66.2	0.783
125	40	none	58.0	43.5	0.633
125	50	none	73.0	34.0	
125	20	20-$NaClO_4$	46.0	36.5	0.349
125	40	20-$NaClO_4$	73.5	26.9	
125	20	20-$Ca(H_2PO_4)_2$	39.0	38.9	0.312
125	40	20-$Ca(H_2PO_4)_2$	67.5	30.0	
125	10	5-$Ca(H_2PO_4)_2$	17.0	78.5	0.973
125	20	5-$Ca(H_2PO_4)_2$	31.5	64.4	0.810
125	40	5-$Ca(H_2PO_4)_2$	60.5	40.9	
135	10	None	12.5	94.5	1.055
135	20	None	25.0	81.3	0.724
135	40	None	50.0	63.2	0.600
135	50	None	62.0	56.0	
135	20	20-$NaClO_4$	39.0	60.0	0.298
135	40	20-$NaClO_4$	62.5	53.0	
135	20	20-$Ca(H_2PO_4)_2$	32.5	60.3	0.265
135	40	20-$Ca(H_2PO_4)_2$	58.5	53.4	
135	10	5-$Ca(H_2PO_4)_2$	15.0	91.5	0.897
135	20	5-$Ca(H_2PO_4)_2$	27.5	80.3	0.802
135	40	5-$Ca(H_2PO_4)_2$	52.5	60.3	

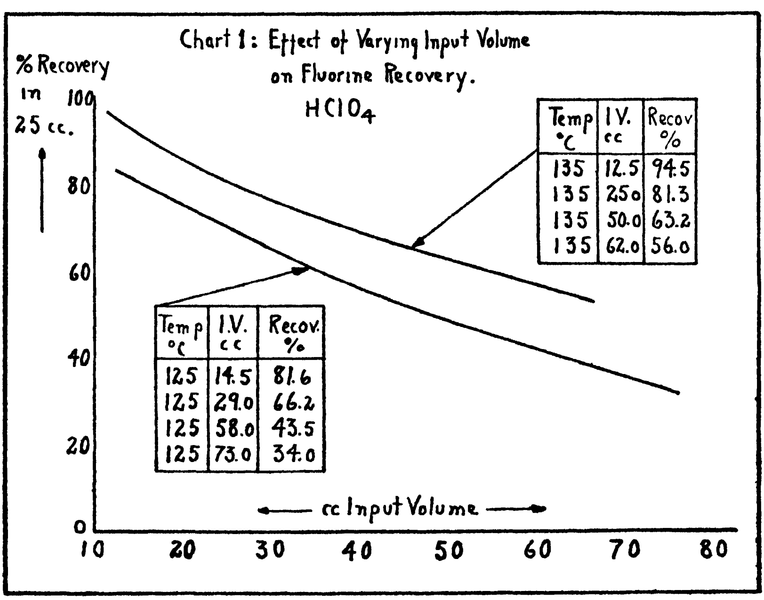
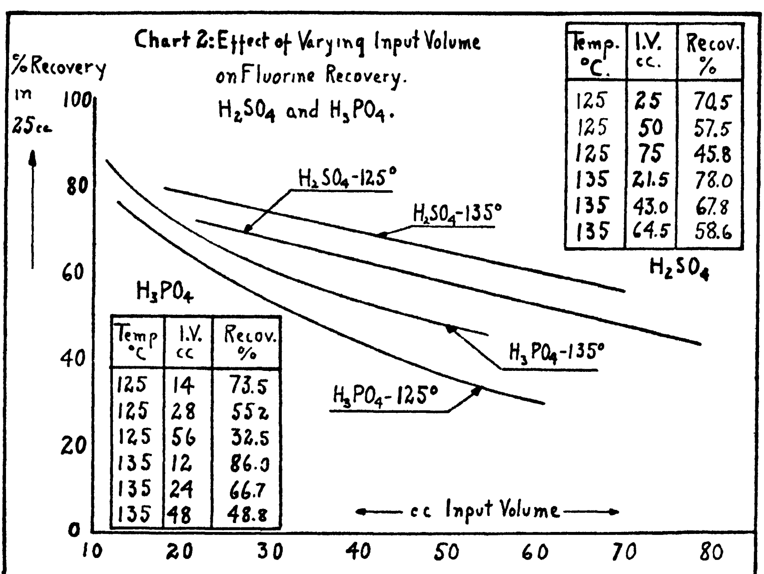


Chart 1 expresses graphically the relation between input volume and recovery at 125° and 135°C. with perchloric acid alone. By reference to this chart it is possible to compare the effect on recovery of a change in the input volume caused by addition of more acid with that caused by the presence of salts. Table 3 summarizes such a comparison. The column "Expected Recovery" contains the values which, according to Chart 1, should have been found at the input volumes stated, had the mixture consisted of perchloric acid and water alone.

TABLE 3.—*Effect of salts on recovery*

TEMP.	SALT USED	I.V.	EXPECTED RECOVERY	FOUND	DIFF.	DIFF. PER 1 G. OF SALT
°C.	*gram*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
125	20-$NaClO_4$	46.0	52.5	36.5	16.0	0.80
125	20-$NaClO_4$	73.5	33.7	26.9	6.8	0.34
125	20-$Ca(H_2PO_4)_2$	39.0	57.8	38.9	18.9	0.95
125	20-$Ca(H_2PO_4)_2$	67.5	37.0	30.0	7.0	0.35
135	20-$NaClO_4$	39.0	70.0	60.0	10.0	0.50
135	20-$NaClO_4$	62.5	55.9	53.0	2.9	0.15
135	20-$Ca(H_2PO_4)_2$	32.5	75.0	60.3	14.3	0.72
135	20-$Ca(H_2PO_4)_2$	58.5	58.0	53.4	4.6	0.23

In like manner the effect on recovery of 5 grams of calcium monophosphate was shown by experiment to be so small as to be within the probable error of the determination.



A similar comparison for sulfuric acid, sulfates, and persulfates appears in Tables 4 and 5 and Chart 2.

TABLE 4.—*Effect of input volume on recovery*

TEMP.	H_2SO_4	SALT	I.V.	RECOVERY	CHANGE IN RECOV. PER CC. I.V.
C°.	*cc.*	*grams*	*cc.*	*per cent*	*per cent*
125	10	None	25	70.5	0.520
125	20	None	50	57.5	0.468
125	30	None	75	45.8	
125	20	15-Na_2SO_4	53.5	51.2	1.58
125	20	30-Na_2SO_4	58.5	43.3	
125	20	15-$Am_2S_2O_8$	61.0	40.0	0.43
125	20	30-$Am_2S_2O_8$	74.0	34.4	

TABLE 5.—*Effect of salts on recovery*

TEMP.	SALT USED	I.V.	EXPECTED RECOVERY	FOUND	DIFF.	DIFF. PER 1 G. OF SALT
°C.	*grams*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
125	15-Na_2SO_4	53.5	56.0	51.2	4.8	0.32
125	30-Na_2SO_4	58.5	53.5	43.3	10.2	0.34
125	15-$Am_2S_2O_8$	61.0	52.5	40.0	12.5	0.96
125	30-$Am_2S_2O_8$	74.0	45.8	34.2	11.6	0.39

From Tables 2–5 it appears—

(1) That the recovery decreases with increasing input volume.

(2) That the change in recovery decreases with increasing volume.

(3) That the presence of soluble salts of non-volatile acids decreases the fluorine recovery more than is indicated by the corresponding increase in input volume. This "retarding effect of soluble salts" becomes important when material low in fluorine is to be analyzed and relatively large quantities of sample must be used.

Table 6 shows a comparison between sulfuric, perchloric, and phosphoric acids in regard to the changes in recovery caused by variations in input volume. In each case the change in input volume (Δ I.V.) resulted from varying the amount of acid used between the limits indicated. The corresponding recoveries as well as the change in recovery (Δ Rec.) are given in this table.

Of particular interest in Table 6 is the comparison of recoveries at equal (or approximately so) input volumes and equal temperatures. Apparently the effect of input volume changes is almost twice as great when perchloric or phosphoric acid is used as with sulfuric acid (0. 78 and 0.81 compared to 0.52, and 0.73 and 0.75 compared to 0.48). This observation is of practical interest when the problem is to get complete recoveries in the smallest possible volume of distillate. Still other comparisons of the three acids may be made by reference to the data in Charts 1 and 2.

TABLE 6.—*Effect of input volume, comparison*

TEMP.	ACID USED	LIMITS	I.V. LIMITS	Δ I.V.	RECOVERIES	Δ REC.	Δ REC./CC. Δ I.V.
°C.		cc.	cc.	cc.	per cent	per cent	per cent
125	H_2SO_4	10–20	25–50	25.0	70.5–57.5	13.0	0.52
125	H_2SO_4	20–30	50–75	25.0	57.5–45.8	11.7	0.47
135	H_2SO_4	10–20	21.5–43	21.5	78.0–67.8	10.2	0.48
135	H_2SO_4	20–30	43–64.5	21.5	67.8–58.6	9.2	0.43
125	$HClO_4$	10–20	14.5–29	14.5	81.6–66.2	15.4	1.06
125	$HClO_4$	20–40	29–58	29.0	66.2–43.5	22.7	0.78
125	$HClO_4$	40–50	58–73	15.0	43.5–34.0	9.5	0.63
135	$HClO_4$	10–20	12.5–25	12.5	94.5–81.3	13.2	1.06
135	$HClO_4$	20–40	25–50	25.0	81.3–63.2	18.1	0.73
135	$HClO_4$	40–50	50-62	12.0	63.2–56.0	7.2	0.60
125	H_3PO_4	10–20	14–28	14.0	73.5–55.2	18.3	1.31
125	H_3PO_4	20–40	28–56	28.0	55.2–32.5	22.7	0.81
135	H_2PO_4	10–20	12–24	12.0	86.0–66.7	19.3	1.61
135	H_3PO_4	20–40	24–48	24.0	66.7–48.8	17.9	0.75

Thus it is apparent that perchloric acid and sulfuric acid cause a more rapid rate of volatilization of fluorine than does phosphoric acid. Comparisons made at equal input volumes show that both sulfuric and perchloric acids are more efficient at 125°C. than phosphoric acid is at 135°. This would be of practical interest in connection with the problem of fluorine removal from commercial phosphoric acid, as well as in the substitution of this acid for the other acids in the distillation procedure.

2. EFFECT OF TEMPERATURE

The effect of varying the temperature at which the distillation is carried out was also studied. Table 7 gives the results.

The "temperature effect" is really the sum of two causes working in the same direction: (1) The decrease in input volume and (2) the increase in temperature. The effect of decreasing input volume can be estimated (at least approximately, *cf.* Table 2). The last column gives the effect of temperature alone.

Apparently the recovery is greater if the distillation is carried out at a higher temperature. The temperature effect per degree is greater when large volumes are present during the distillation than with small volumes.

At this point it is well to stress the explosive character of anhydrous perchloric acid, particularly in the presence of even small quantities of organic matter. Table 1 indicates that above 142°C. concentration of the 60 per cent acid begins. Distillations above this temperature, therefore, are likely to become increasingly dangerous.

Table 6 also shows a comparison of the three acids with regard to the temperature effect at similar input volumes. It seems to be about twice as great for perchloric as for sulfuric acid (1.13 and 1.46 as compared to 0.57 and 0.71), while phosphoric acid appears to occupy an intermediate position (0.84 and 1.13).

TABLE 7.—*Effect of distillation temperature on recovery*

TEMP.	ACID	I.V.	Δ I.V.	RECOV.	Δ RECOV.	Δ RECOV./1°C. TOTAL	Δ RECOV./1°C. CORRECTED FOR I.V.
°C.	cc.		cc.	per cent	per cent	per cent	per cent
125	10-$HClO_4$	14.5	2.0	81.6	12.9	1.29	1.05
135	10-$HClO_4$	12.5		94.5			
125	20-$HClO_4$	29.0	4.0	66.2	15.1	1.51	1.13
135	20-$HClO_4$	25.0		81.3			
125	40-$HClO_4$	58.0	8.0	43.5	19.7	1.97	1.46
135	40-$HClO_4$	50.0		63.2			
125	50-$HClO_4$	73.0	11.0	34.0	22.2	2.22	1.59
135	50-$HClO_4$	62.0		56.2			
125	10-H_3PO_4	14.0	2.0	73.5	12.5	1.25	0.96
135	10-H_3PO_4	12.0		86.0			
125	20-H_3PO_4	28.0	4.0	55.2	11.5	1.15	0.84
135	20-H_3PO_4	24.0		66.7			
125	40-H_3PO_4	56.0	8.0	32.5	16.3	1.63	1.13
135	40-H_3PO_4	48.0		48.8			
125	10-H_2SO_4	25.0	3.5	70.5	7.5	0.75	0.57
135	10-H_2SO_4	21.5		78.0			
125	20-H_2SO_4	50.0	7.0	57.5	10.3	1.03	0.71
135	20-H_2SO_4	43.0		67.8			
125	30-H_2SO_4	75.0	9.5	45.8	12.7	1.27	0.86
135	30-H_2SO_4	64.5		58.6			

3. VARIATION WITH OUTPUT VOLUME

For fluorine distillations with sulfuric acid the quantitative recovery varied with the amount of distillate collected, according to the following equation:

$$K=\frac{1}{t}\log\frac{C}{C-X}.$$

Here K is a constant, t equals the number of cc. distillate collected, C represents the orginal concentration of fluorine and $(C-X)$ the concentration remaining in the flask after t cc. has been distilled. If C and X are

TABLE 8.—*Variation of recovery with amount of distillate*

ACID USED	RECOVERY IN FIRST 25 CC.	RECOVERY IN SECOND 25 CC. FOUND	RECOVERY IN SECOND 25 CC. CALCULATED FROM FIRST 25 CC.
	per cent	per cent	per cent
$HClO_4$	54.3	22.0	24.8
$HClO_4$	52.8	24.5	24.9
$HClO_4$	53.1	25.7	24.9
$HClO_4$	57.0	24.4	24.5
$HClO_4$	85.0	13.8	12.8
H_3PO_4	48.5	25.5	25.0
H_3PO_4	33.0	22.1	22.3

expressed in percentage concentrations, C becomes 100 and X becomes the percentage recovery in t cc.

For distillations with perchloric and phosphoric acids, checks on the validity of this formula, made by recovering separately (at constant temperature) the first 25 cc. of distillate and the next 25 cc. and determining the fluorine in each solution, gave the results shown in Table 8.

These results (Table 8) indicate that the distillations with perchloric and phosphoric acids follow the same general principles as does the distillation with sulfuric acid.

SUMMARY

A study was made of certain factors influencing the recovery of fluorine by distillation with perchloric and phosphoric acids. It appears—

(1) That the recovery per cc. distillate decreases with increasing volume of liquid in the distilling flask.

(2) That distillations carried out at higher temperature give increased recoveries per cc. of distillate.

(3) That these variations in the recovery rate are greater when distillations are made with perchloric or phosphoric acid, than with sulfuric aicd.

(4) That the addition of soluble salts of non-volatile acids to the system in the distilling flask causes a greater decrease in the recovery rate than would be expected from the increase in volume caused by the presence of such salts.

(5) That the recovery for both perchloric and phosphoric acids varies with the amount of distillate collected, according to the logarithmic equation previously found to apply to distillations with sulfuric acid.

THE COLORIMETRIC DETERMINATION OF LACTIC ACID IN TOMATO PRODUCTS

By FRED HILLIG (Food Division,* Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

The lactic acid content of certain foods may be significant to the regulatory chemist in detecting sophistication and also as an index of any retrograde changes involved in decomposition. Accordingly, it seemed desirable to test the applicability of the writer's colorimetric method for the determination of lactic acid in milk and milk products[1] to certain other foods especially susceptible to those types of decomposition likely to produce lactic acid. Tomato products at once suggested themselves because Bacon and Dunbar[2] found that sound tomatos contain little or no lactic acid, but that formation of this acid is associated in some instances with the spoilage of tomatoes.

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* W. B. White, Chief.
[1] *This Journal*, **20**, 130 (1937).
[2] U. S. Bur. Chem. Cir. 78.

It was found that with a few modifications the method cited could be readily applied to the determination of lactic acid in tomato products. The principal steps in the proposed procedure are (1) extraction of the lactic acid, (2) purification of the extracted acid with carbon, and (3) development and final estimation of lactic acid colorimetrically.

The original paper gives detailed information regarding the extractor used, reasons for the carbon treatment, the mechanics of the development of the characteristic yellow color with ferric chloride, and the final estimation of lactic acid either photometrically or in Nessler tubes. However, a filter centering around 460 mμ instead of one centering around 450 mμ was used in making the photometric comparisons because it was thought it might not be possible with all photometers to place a piece of Noviol Yellow glass in the light beam traversing the wedge of the instrument. With filter 460 mμ the hue difference is greater than with filter 450, but it is believed that the analyst should have no difficulty in making good readings.

LACTIC ACID IN TOMATO PRODUCTS

REAGENTS

Same as those given in the original paper.

DETERMINATION

Weigh 20 grams of tomato paste, catsup, or chili sauce (in case of tomato juice use 40 g.) into a small beaker and transfer to a 200 cc. volumetric flask with water. Make to mark, shake, and filter. If filtration is slow, centrifuge the material, decant into a flask, add a small amount of filter cel, shake, and filter. Transfer 50 cc. of the filtrate to the continuous extractor, add 1 cc. of H_2SO_4 (1+1) and proceed with the determination as directed in the previous paper, except to extract 3 hours instead of 2 hours. With catsup or other products containing acetic acid, after extraction and evaporation of the ether, make to 50 cc. and steam distil to eliminate the acetic acid. (After 250 cc. of distillate has been collected the material is sufficiently free of acetic acid.) Evaporate to 20 cc., neutralize with a saturated $Ba(OH)_2$ solution, transfer to a 110 cc. volumetric flask with alcohol, and proceed as directed in the original method.

In order to test the accuracy of the method, varying quantities of lactic acid as lithium lactate were added to good quality tomato juices. Recoveries obtained by the proposed method are shown in Table 1.

It will be seen (Table 1) that closely agreeing duplicate results were obtained and that the method recovers practically all the lactic acid added. The fact that the "blank" is an important fraction of the added lactic acid at the 1 mg. level may furnish less justification for calculating "per cent recovery," than when 5 mg. or more lactic acid is added.

Because with catsup or other products containing acetic acid it is necessary to steam distil after extraction, experiments were conducted with a view to ascertaining whether or not there is any loss of lactic acid during the distillation. Varying quantities of lactic acid as lithium lactate were added to catsups. The recoveries by the proposed method are shown in Table 2.

TABLE 1.—*Recovery of lactic acid added to tomato juice by the proposed method*

LACTIC ACID ADDED	LACTIC ACID DETERMINED	LACTIC ACID IN JUICE (10 G.)	LACTIC ACID RECOVERED	
mg.	*mg.*	*mg.*	*mg.*	*per cent*
	1.48		0.94	94.0
1	1.51	0.54	0.97	97.0
	5.03		4.65	93.0
5	5.17	0.38	4.79	95.8
	10.24		9.78	97.8
10	10.36	0.46	9.90	99.0
	14.81		14.45	96.3
15	15.09	0.36	14.73	98.2
	20.21		19.68	98.4
20	20.03	0.53	19.50	97.5
	25.25		24.48	97.9
25	25.44	0.77	24.67	98.7
	29.79		29.18	97.3
30	29.76	0.61	29.15	97.2
	40.74		39.96	99.9
40	40.34	0.78	39.56	98.9
	50.00		49.44	98.9
50	49.10	0.56	49.54	97.1
	59.38		58.60	97.7
60	58.63	0.78	57.85	96.4
	72.08		71.57	95.3
75	70.86	0.51	70.35	93.8

TABLE 2.—*Recovery of lactic acid added to catsup by the proposed method*

LACTIC ACID ADDED	LACTIC ACID DETERMINED	LACTIC ACID IN CATSUP (5 G.)	LACTIC ACID RECOVERED	
mg.	*mg.*	*mg.*	*mg.*	*per cent*
	6.13		4.90	98.0
5	5.97	1.23	4.74	94.8
	11.15		9.71	97.1
10	11.31	1.44	9.87	98.7
	24.99		23.71	94.8
25	25.53	1.28	24.25	97.0
	49.61		48.17	96.3
50	49.97	1.44	48.53	97.1
	72.64		71.20	95.0
75	71.75	1.44	70.31	93.6

Lactic acid was determined in authentic tomato juices by the proposed method, and the results are given in Table 3.

TABLE 3.—*Lactic acid in authentic tomato juices determined by the proposed method*

SAMPLE NO.	LACTIC ACID	SAMPLE NO.	LACTIC ACID
	mg/100 g.		*mg/100 g.*
	7.8		6.0
1	7.5	8	7.2
	3.8		6.2
2	3.8	9	6.0
	5.4		
3	5.4	10	8.0
			7.6
	5.1		
4	5.0		5.6
		11	5.6
	4.7		
5	4.5		8.3
		12	7.2
	3.3		
6	3.8		5.6
		13	6.0
	3.3		
7	3.8		

Lactic acid was determined in some commercial tomato products by the proposed method, and the results are given in Table 4.

TABLE 4.—*Lactic acid in commercial tomato products determined by the proposed method*

MATERIAL	LACTIC ACID	MATERIAL	LACTIC ACID
	mg/100 g.		*mg/100 g.*
	24.8		25.4
Catsup	26.2	Paste	23.2
	25.6		20.6
Catsup	25.6	Paste	19.8
	25.6		5.5
Catsup	23.6	Pulp	5.5
	29.0		21.2
Catsup	28.4	Chili Sauce	21.8
	25.4		25.6
Catsup	26.2	Chili Sauce	27.2
	25.6		
Catsup	24.8		

In all the tables the expression "lactic acid," in the cases where none was added, is to be interpreted as material reacting as lactic acid under the method of analysis. The difficulty of establishing that a quantity of material of such small magnitude is, in fact, lactic acid is obviously very great.

SUMMARY

A method applicable to tomato products is proposed for the colorimetric determination of lactic acid. The various steps in the procedure are not difficult, and the determination can be completed in a working day.

STUDIES ON THE PROTEOLYTIC ACTIVITY OF BARLEY MALT

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York City)

Considerable significance is attached to the breakdown of barley and malt proteins by the proteolytic enzymes present in malt during the malting and mashing process, because it is recognized that the resulting nitrogenous substances greatly influence the body, flavor, and general character of the beer. In spite of the numerous investigations on the extent of this action and recent progress in studying the nature and action of enzymes in general, and of proteolytic enzymes of barley malt in particular, the available knowledge concerning this activity and especially of satisfactory methods of measuring it is still incomplete.

The existence of a proteolytic enzyme in germinating barley was first discovered by Gorup-Besanez,[1] who found that a malt infusion acting on blood fibrin yielded soluble products that exhibited reactions usually considered characteristic of peptic digestion. Griessmayer[2] gave this ferment the name "peptase." Its importance in brewing in regard to "peptonization" of proteins during malting and mashing was recognized at that early date; it also was found to increase in activity in the presence of acids. R. Wahl,[3] in 1893, even assumed the action of peptase on proteins to be analogous to that of diastase on starch in the course of brewing. Since its discovery numerous workers (see H. Lüers)[4] have attempted to detect the presence of peptase in germinating grain with varying results. In 1900 Fernbach and Hubert,[5] Petit and Labourasse,[6] and Windisch and Schellhorn,[7] working independently of each other, established the existence of proteolytic enzymes in malt by experimental means, such as auto-digestion of aqueous malt extracts, liquefaction of gelatin, and preparation of an active proteolytic substance by extraction with glycerol.

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[1] *Ber.*, 7, 1478 (1874).
[2] Thausing, Malzbereitung und Bierfabrikation, 177 (1882).
[3] *Am. Brewers' Rev.*, 7, 201 (1893).
[4] Chemie des Brauwesens, p. 208 (1929).
[5] *Compt. rend.*, 130, 1787 (1900).
[6] *Ibid.*, 131, 349 (1900).
[7] *Wochschr. Brau.*, 27, 334 (1900).

It also was demonstrated by Fernbach and Hubert[1] that neutralization of the secondary phosphates of a malt infusion with phosphoric acid to methyl orange causes a considerable increase in proteolytic activity. The investigations that followed during that early period were concerned with characterization and classification of the proteolytic enzymes of malt. Weiss[2] differentiated between "peptase" and "tryptase" found in malt, and also noted an increase in activity on addition of a small amount of acids. Schjerning,[3] by means of fractional precipitation methods, also was able to distinguish peptic from tryptic action of malt. Adler,[4] on the other hand, working with edestin, gelatin, and Witte's peptone as substrates and using formol titration for measuring the extent of proteolysis, found the existence of only one enzyme with optimum activity at *p*H 4.3–5.0. Lundin[5] tested the liquefying effect of malt infusions on gelatin, reporting an optimum *p*H 3.1–3.4 for an enzyme that he designated as peptase. He further studied the increase in formol nitrogen with Witte's peptone and found an optimum *p*H 6.2–6.4 for what he called tryptase. To that period also belong the first attempts to determine the proteolytic activity of malts. Credit is due to Schidrowitz,[6] who modified Windisch's original gelatin procedure. The method resembled Lintner's original test tube procedure for estimating the diastatic power of malts. Increasing amounts of malt infusions were added to the same quantity of a gelatin solution in test tubes, which were incubated for 20 hours at 40°C.; these then were cooled for 1 hour at 15°C., the lowest number of cc. of the malt infusion which prevented the re-setting of the gelatin being noted. This figure was divided into 100 and called the proteolytic power of the malt in question. Schidrowitz later recognized the difficulty of obtaining samples of gelatin sufficiently uniform to give reproducible results. The Schidrowitz method, with some modifications, was used by R. Wahl[7] for studies on the proteolytic enzyme and proteolytic activity of malts. Based on former experiments by Nilson,[8] Wahl found that malt infusions prepared with water acidified with small amounts of bacterial lactic acid (0.1–2 per cent) exerted a considerably higher liquefying power on gelatin than did aqueous extracts, while commercial lactic acid and inorganic acids yielded practically no increase in proteolytic activity. As a standard of comparison Wahl introduced diluted solutions of pepsin of 1:10,000 strength, which were permitted to act upon gelatin under the same conditions that prevailed during the malt infusions. The same author[9] recently modified his method somewhat. Later another modification was published by A. S. Wahl,[10] who introduced papain as a standard of comparison.

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[1] *Compt. rend.*, 131, 293 (1900).
[2] *Compt. rend. lab. Carlsberg*, 5, 135 (1903).
[3] *Ibid.* 9, 319 (1914).
[4] *Z. ges. Brauw.*, 38, 129 (1915).
[5] *Biochem. Z.*, 131, 193 (1922).
[6] *J. Inst. Brewing*, 19, 361 (1903); 25, 592 (1909).
[7] *Eighth Int. Congr. Appl. Chem.*, 1912, Section VI b, 215.
[8] *Am. Brewers' Rev.*, 18, 294 (1904).
[9] Commuo. Master Brewers' Assoc. of America, 6, July, p. 9 (1935).
[10] *Am. Brewers' Rev.*, 50, July, p. 21 (1936).

Two other procedures that have been suggested for measuring the proteolytic strength of malts deserve mention. C. A. Nowak[1] estimated the acid ratio, *i.e.*, the relationship of amino acids (formol titration) to that of the normal acidity (regular titration) of a malt infusion; a high ratio indicates high activity. L. Idoux,[2] on the other hand, allowed a malt infusion to act on natural barley proteins (barley flour) in which the enzymes had been destroyed by drying for 4 hours at 105°C. The increase in nitrogen as compared with a blank, where the barley flour was treated in a similar manner with a boiled portion of the malt infusion, was used as a measure of the proteolytic power of the malt in question.

Due to investigations of Willstätter and his co-workers,[3] conceptions regarding plant proteolytic enzymes have changed in recent years. It was recognized that the earlier classification of plant enzymes into "peptase," derived from animal pepsin, and "tryptase," derived from animal trypsin, was no longer tenable. It is now realized that the plant enzymes must first be subjected to a thorough study, with modern technic, before a suitable classification can be proposed. For this reason, those plant enzymes that hydrolyze natural proteins, for the time being are called proteinases. They are most active at the isoelectric point of the respective proteins, the optimum *p*H of their activity varying with the substrate. They appear to be related to papainases, both groups of enzymes being activated with hydrocyanic acid. At the present time, the proteolytic enzymes capable of splitting peptides into amino acids are termed peptidases.

Several recent studies on proteolytic enzymes of malt conducted from this modern point of view are available. Mill and Linderström-Lang[4] established in malt infusions the presence of a proteinase acting upon edestin (optimum *p*H 4.3), gelatin, and Witte's peptone. Lüers and Malsch[5] also observed a proteinase in malt infusions and reported its optimum activity on gelatin at *p*H 4.9–5.0, using Willstätter's method of alcoholic titration of carboxyl groups liberated in the course of digestion. Hopkins and co-workers[6] measured the extent of proteolysis by determining the amount of nitrogen that was rendered soluble and non-coagulable by the action of malt infusions on crystalline egg albumin at *p*H 4.6 and by formol titration of Witte's peptone. Mounfield[7] found in germinating wheat a similar enzyme showing its optimum action on edestin at *p*H 4.1. In addition, all these investigators established the existence of one or more peptidases acting on dipeptides leucylglycine and glycylglycine at *p*H 7.5–8.6, the presence of erepsin (peptidase) in germinating barley having been demonstrated by Abderhalden and Dammhahn[8] as early as 1909.

[1] *Ind. Eng. Chem.*, 7, 858 (1915).
[2] *Brasserie Malterie*, 23, 122 (1933).
[3] *Z. physiol. Chem.*, 151, 307 (1926).
[4] *Compt. rend. trav. lab. Carlsberg*, 17, No. 10, 1 (1929).
[5] *Wochschr. Brau.*, 46, 265 (1929).
[6] *Biochem. J.*, 23, 1022 (1929); *J. Inst. Brewing*, 36, 9 (1930).
[7] *Ibid.*, 30, 549, 1778 (1936).
[8] *Z. physiol. Chem.*, 57, 332 (1909).

When the experimental part of the investigation presented here was completed, a paper by Kolbach and Simon[1] was published, and in some respects it shows similarities to the studies presented in this paper. These authors suggest a method for evaluating the proteolytic activity of malts depending upon the determination of the amount of nitrogen rendered soluble and non-coagulable, when malt infusions are allowed to act upon edestin for 6 hours at pH 4.5 at 35°C. They state that maximum extraction of the proteolytic enzymes occurs at pH 4.5 when the mash is held for 30–60 minutes at 20°C. The proteolytic enzymes present in malt are not completely extractable, and for this reason the proteolytic activity of a malt infusion does not represent the proteolytic power of a malt. The latter can be estimated approximately by the method of Lüers and Loibl,[2] the extent of proteolysis being measured by determining the formol nitrogen produced in a mash held for 3 hours at 50°C., as compared with a control mash in which the enzymes have been destroyed with alcohol treatment. Kolbach and Simon,[1] using their edestin method on 8 different malts, and also a modification of the Lüers and Loibl comparative cold and warm mashes, showed that there is no relationship between the proteolytic strength of a malt infusion and the proteolysis occurring during mashing.

The purpose of the present investigation was to determine the rate of proteolysis of infusions from different malts, when several substrates are employed. It was assumed that this would give some information as to the nature of the proteolytic enzymes present in malt, and would permit a comparison between the proteolytic activity of a malt infusion and that which takes place in a laboratory mash. For this purpose 10 malts of various origin were selected. The results are presented in Tables 6 to 10.

METHODS AND RESULTS

GENERAL ANALYSIS

The malts were analyzed in the regular manner by the methods adopted by the American Society of Brewing Chemists[3] (Tables 6 and 7). A slightly modified procedure was used for estimating their Lintner value. The infusions were prepared by extracting for 1 hour at 40°C. A reduction was carried out on the digestion mixtures and blanks by the Munson-Walker general method,[4] and from the cuprous oxide found the corresponding maltose hydrate values were read from the tables. These maltose values, after deduction of the blanks, were used as a basis for computing the Lintner values. According to the Lintner scale, a malt has 100° if 0.1 cc. of a filtered 5 per cent infusion produces enough reducing sugars to just reduce 5 cc. of Fehling's solution under definite conditions; or 1 cc. of the malt infusion would reduce 50 cc. of Fehling's solution containing 438 mg. of

[1] *Wochschr. Brau.*, 53, 297, 310 (1936).
[2] *Z. ges. Brauw.*, 46, 29 (1923).
[3] *Official Methods for Analysis of Malt* (1935).
[4] *Methods of Analysis, A.O.A.C.*, 1930.

copper, which is equivalent to 493.1 mg. of cuprous oxide and to 408.3 mg. of maltose. The diastatic power of a malt would then be

$$\frac{\text{mg. maltose} \times 100}{408.3 \times A},$$

where A represents the number of cc. of undiluted malt extract used for digestion; if A is 1.0 cc. and the 50 cc. of Fehling's solution is just reduced, the diastatic power is 100. In the present experiments 2 cc. was used to act upon 100 cc. of a 2 per cent starch solution for a half-hour at 20°C.; the digestion mixture was then brought to a volume of 200 cc., and 50 cc. was used for reduction. To calculate to mg. of maltose corresponding to 200 cc., and for 1 hour's digestion according to Lintner, the formula would be:

$$\frac{\text{mg. maltose} \times 100}{408.3 \times 2} \times 4 \times 2 = \text{mg. maltose} \times 0.97967.$$

It is realized that this method of computing Lintner values yields results that differ somewhat from those obtained by other standard methods. However, the use of maltose values as a basis of calculation has the advantage that it permits ready conversion to other forms of expressing the diastatic power of a malt; for instance, in grams of maltose produced under definite conditions (half-hour digestion at 20°C.) by 100 grams of malt; this may be found from the formula:

$$\frac{\text{mg. maltose} \times 4 \times 1000}{1000} = \text{mg. of maltose} \times 4.$$

By multiplying the Lintner figure by 4.083 and the grams of maltose by 0.24492, the values may be converted into each other. Both values were also recorded for the malts tested.

The laboratory mash (Table 7) refers to the regular mashing procedure described in the methods for malt analysis. In the cold water mash (Table 8) the same proportion of finely ground malt and water was used as in the laboratory mash; however, water cooled to 3°C. was used for mashing, and the mash was maintained at this temperature with frequent stirring for 1 hour. After being brought to the required weight, the mash was filtered in the ice box, where the filtered cold water extract was kept all the time. Portions necessary for analysis were withdrawn and immediately subjected to the treatment required, and the analysis was completed on the same day. This procedure prevented any marked enzymic action from taking place in the cold mash.

The nitrogen determinations were made by the Kjeldahl-Gunning method.[1] By soluble nitrogen is meant the nitrogen rendered soluble either during mashing (laboratory mash) or during extraction in the cold (cold mash); it was estimated in the respective worts. For determination of the permanently soluble nitrogen the worts were boiled down to half

[1] *Methods of Analysis, A.O.A.C.*, 1930.

the volume and kept in ebullition for 2 hours, boiling water being added frequently to keep the volume constant; then they were brought to the original volume at 20°C. and filtered through folded filter paper. A nitrogen determination was made on the filtrate, which was also used for estimation of formol nitrogen and of fermentable extract by the methods described in Pawlowski-Doemens.[1] The nitrogen figures were calculated to the basis of 100 grams of dry malt by multiplying the values found in the respective worts by $Y/E \times S$, in which S indicates the specific gravity, E the corresponding extract in per cent (Plato) of the wort examined, and Y the yield of extract, dry basis, as computed from the former values and the moisture content of the malt. The *p*H determinations were made by the glass electrode.

DETERMINATION OF PROTEOLYTIC ACTIVITY

Various methods were tested for measuring the proteolytic activity of malt infusions. The Idoux method gave irregular results; likewise, great difficulty was experienced with the Schidrowitz procedure as modified by Wahl. Neither of these methods yielded reproducible values, although with the latter in some instances some information could be obtained as to relative proteolytic activity of the malts tested. It was thought desirable to use one method to investigate the initial stage of proteolysis, and another to obtain information regarding the further degradation into simple peptides and amino acids. The use of edestin as a substrate, and determination of both permanently soluble nitrogen and amino nitrogen in form of free carboxyl groups, offered possibilities. Finally three methods were selected.

Viscometric Method

This procedure as developed by Northrop and Hussey[2] is well suited for measuring that component of proteolytic enzymes that exerts the initial fission, the one that disaggregates the protein molecule or breaks it down into smaller aggregates. Lüers and Löther[3] made some preliminary tests on malt infusions with this procedure. The method depends upon measuring, under definite conditions, the changes in viscosity of a gelatin solution effected by a proteolytic enzyme. It has been observed that the time required to cause a definite percentage change in the initial viscosity of the substrate is inversely proportional to the amount of enzyme employed; double the amount requires half the time to produce the same change, etc. (Arrhenius "Q×T" rule). Thus it is possible to define the activity of an enzyme in terms of the time required to effect a definite change in the initial viscosity of the substrate. It was found that this holds true also for the proteolytic activity of malt infusions when the

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[1] Die Brautechnischen Untersuchungs-Methoden (1932).
[2] *J. Gen. Physiol.*, 5, 353 (1923).
[3] *Woch. Brau.*, 52, 49 (1935).

tests are carried out under definite conditions; the values obtained readily can be expressed in units, and the results are reproducible.

For making the tests,[1] U.S.P. granulated gelatin was washed at 3°C. twice with 20 times its weight of water, and twice with the same amount of N/128 acetic acid to bring it to its isoelectric point of *p*H 4.7. After decantation of the supernatant liquid, the semi-solid material was poured on a Büchner funnel over which one thickness of muslin had been spread, and excess fluid was removed by suction. The gelatin was then placed in a flask and evaporated in a water bath to 15 per cent concentration as ascertained by drying tests. The substrate thus prepared was distributed in small stoppered flasks and preserved in a refrigerator with addition of 0.5 per cent toluene.

For viscosity determinations 25 grams of this gelatin solution was withdrawn, made up with 10 cc. of 0.2 *M* Sörenson's citrate buffer of *p*H 5.0 and with water to 100 cc. (3.75% concentration), and filtered through quantitative filter paper to remove suspended material, especially cotton fibers, which were found to interfere with the test. Ostwald viscometers having 60–80 seconds outflow time for 5 cc. of water at 40°C. were

TABLE 1.—*Viscosity test with the full and the half quantity of a malt infusion*

FULL QUANTITY (5 CC.)			HALF QUANTITY (2.5 CC.)		
TIME OF OBSERVATION AFTER ZERO TIME	OUTFLOW TIME	LOGARITHM	TIME OF OBSERVATION AFTER ZERO TIME	OUTFLOW TIME	LOGARITHM
minutes	*seconds*		*minutes*	*seconds*	
3:	241.7	0.38328	2:	199.4	0.29973
7:30	237.8	.37621	6:30	198.3	.29732
12:30	235.0	.37107	12:00	197.2	.29491
17:30	232.9	.36717	24:30	194.2	.28825
22:30	230.1	.36192	38:30	191.2	.28149
27:30	227.7	.35736	48:30	189.2	.27692
32:30	226.5	.35507	53:30	188.1	.27439
37:30	273.3	.34889	58:00	186.9	.27161
42:30	221.3	.34498	62:30	186.1	.26975
47:30	219.5	.34143	67:	185.5	.26834
52:30	217.8	.33806	71:	184.7	.26647
57:30	216.0	.33445	75:30	184.2	.26529
			80:	183.2	.26293
			84:	182.6	.26150
			88:	181.5	.25888
			92:30	181.0	.25768
			97:	180.6	.25672
			101:30	179.9	.25503
			110:30	178.2	.25091

used. The time ratio of the gelatin-malt infusion mixture to water was approximately 3:1. Twenty cc. of the filtered gelatin solution and the

[1] S. A. WAKSMAN and W. C. DAVISON, Enzymes, p. 210 (1926).

malt infusion were attemperated separately in a water bath for 15 minutes at 42°C.; 5 cc. of the malt infusion was then added to the gelatin solution (zero time) and mixed thoroughly (final concentration of gelatin 3%), and 5 cc. of the mixture was pipetted promptly into the viscometer, which had previously been attemperated for 15 minutes in a continuously stirred glass water bath held at 40°C. (±0.05).

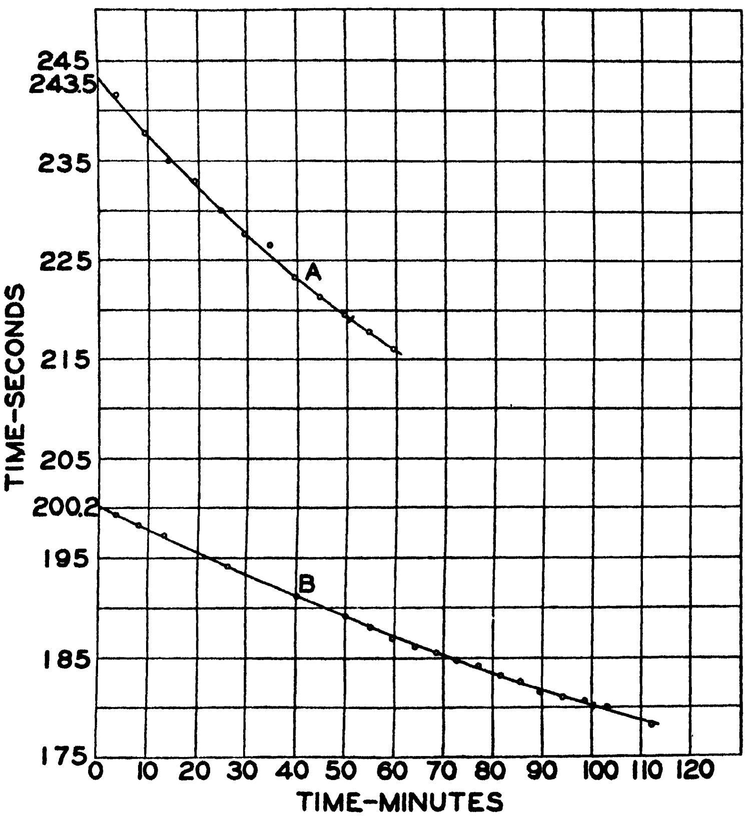

FIG. 1.—CHANGE IN VISCOSITY OF A GELATIN-MALT INFUSION MIXTURE—PLAIN CURVES. A. FULL QUANTITY OF MALT INFUSION; 10% CHANGE 51 MINUTES. B. HALF QUANTITY OF MALT INFUSION; 10% CHANGE 100 MINUTES

Immediately (2–3 minutes after zero time) the first viscosity test was made, and thereafter measurements were taken in frequent succession for about 1 hour or longer, until an approximate 10 per cent change in outflow time of the first reading was obtained. The outflow times were then plotted as ordinates against the time intervals of observation as abscissae; in order to account for changes in viscosity taking place during the measurement, half the outflow time was added to the time each measurement was taken, and the value obtained was recorded as the time

of observation. A smooth curve was drawn through the points, and the time required to effect a 10 per cent change in the initial viscosity (zero time) was read from this curve.

As in the experiments by Northrop, difficulties were encountered in determining the initial outflow time. Attempts to replace the active malt

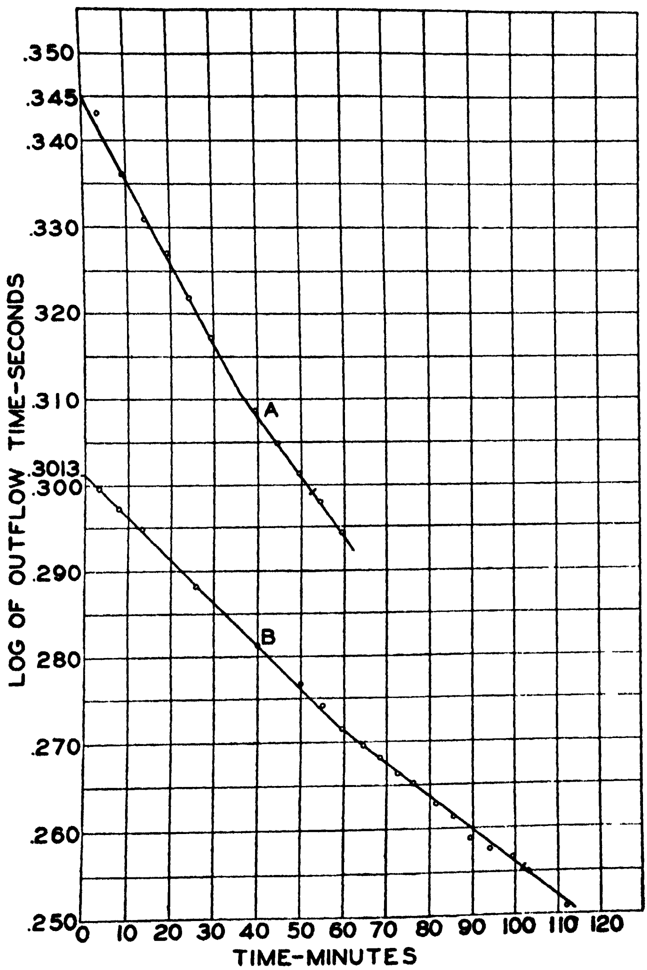

FIG. 2.—CHANGE IN VISCOSITY OF A GELATIN-MALT INFUSION MIXTURE—SEMI-LOGARITHMIC CURVES. A. FULL QUANTITY OF MALT INFUSION; 10% CHANGE 52½ MINUTES. B. HALF QUANTITY OF MALT INFUSION; 10% CHANGE 102 MINUTES.

infusion by an equal volume of water or boiled malt infusion were unsuccessful, and finally the value had to be found by extrapolating the curve. It also was observed that upon plotting the logarithms of the outflow times versus the time intervals of observation, several intersecting straight

lines resulted. By extrapolating the first straight line to zero time, the logarithm of the initial outflow time was secured; the antilogarithm of this value gave the initial outflow time.

Data for a viscosity test with the full (5 cc.) and the half quantity (2.5 cc.) of a malt infusion (the other half quantity having been replaced by water) are presented in Table 1, and graphically in Fig. 1 for the plain, and in Fig. 2 for the semi-logarithmic curves; in order to place the logarithmic curve for the full quantity on the same graph with that for the half quantity, 0.0400 was deducted from all logarithmic values of the measurements for full quantity. Fig. 1, A, plain curve for the full quantity, shows the initial outflow time to be 243.5 sec.; 10 per cent change $243.5\times0.9=219.2$, which is found to be reached within 51 minutes. Plain curve B for half quantity has an initial reading of 200.2, 10 per cent change of which is 180.2 at 100 minutes. In like manner, Fig. 2, A, semi-logarithmic curve for the full quantity shows an initial reading of $0.34500+0.04000=0.38500$, anti-logarithm 242.7 sec. initial outflow time; 10 per cent change $242.7\times0.9=218.4$; the corresponding logarithm is $0.33925-0.04000=0.29925$ at 52.5 minutes. Curve B on Fig. 2 indicates the semi-logarithmic graph for the half quantity with an initial reading of 200.1 and a corresponding 10 per cent change of 180.1 at 102 minutes. These results are recorded in Table 2. It is apparent that the half quantity of a malt infusion corresponding to half enzyme concentration requires almost twice the time to cause the same percentage change in viscosity as the full amount or enzyme concentration; both the plain and the semi-logarithmic curves give fairly close results. A similar degree of precision was obtained on infusions of the same malt when prepared at different times. Although the same gelatin solution once prepared was used for all malts tested, a few preliminary tests with gelatins of various origin indicated that when treated in the same manner they might yield similar results:

MALT NO.	GELATIN	10% CHANGE
		minutes
1	U.S.P. granulated	58
1	Gold label	56
2	U.S.P. granulated	46
2	Silver label	48

The method is promising and with slight modifications in technic (change in construction of viscometer to insure faster flow, change in reaction temperature to 34°C. to prevent thermal effects) offers possibilities of measuring the disaggregating component of malt proteinase with a satisfactory degree of precision. Further work along these lines is in progress.

Conversion to Proteolytic Activity Units.—As a unit of proteolytic activity based on viscosity measurements under specified conditions, there is suggested that amount of enzyme that requires 10 minutes to

TABLE 2.—*Comparison of results from Figures 1 and 2*

	INITIAL READING	10% CHANGE	TIME REQUIRED TO REACH 10% CHANGE
	seconds	*seconds*	*minutes*
	Plain Graph		
A Full Quantity	243.5	219.2	51
B Half Quantity	200.2	180.2	100
	Logarithmic Graph		
A Full Quantity	242.7	218.4	52.5
B Half Quantity	200.1	180.1	102

reduce the initial viscosity 10 per cent. In the present experiments 5 cc. of a 20 per cent malt infusion was mixed with 20 cc. of gelatin solution; from this mixture 5 cc. (containing 1 cc. of malt infusion) was pipetted into the viscometer. Depending on the moisture content of the malts tested and the specific gravity of the infusion, 1 cc. of the malt infusion corresponded to different amounts of dry malt (Table 9). For example, Malt No. 1, domestic 6-rowed, required 46 minutes for 10 per cent change, when 1 cc. of its infusion corresponding to 0.2257 gram of dry malt was allowed to act on the gelatin solution; this indicates 10/46 units in 0.2257 grams, and in 100 grams of dry malt $10/46 \times 100/0.2257 = 96.3$ units.

Edestin Titration Method

For measuring the proteolytic activity of the malt infusions in regard to their capacity of performing "true" proteolysis, the infusions were allowed to act upon edestin as a substrate, and the extent of hydrolysis was determined by the Willstätter titration in alcoholic solution of the carboxyl groups liberated from peptide linkages in the course of digestion. While all gelatin tests were carried out at a *p*H of 5.0, which is the optimum reaction for that substrate, and also not remote from that of a brewer's mash (*p*H 5.1–5.4), the experiments with edestin had to be conducted at a *p*H of 4.3–4.4, this being the approximate optimum for this substrate. When acetic acid was tried, as recommended by Mounfield, for dissolving the edestin and adjusting to the proper hydrogen-ion concentration, large quantities of acid were required to bring the reaction to *p*H 4.3, and neither at *p*H 4.3 nor at *p*H 5.0 could reproducible results be secured. Additions of the buffer solution at *p*H 5.0, regardless of whether acetic or hydrochloric acid was used, caused a considerable precipitation of the substrate, which continued to separate out during the digestion period. It was then decided to adjust the reaction with hydrochloric acid to yield on mixing with the malt infusion a *p*H of 4.3–4.4; following the investigations by Mounfield, a 2 per cent substrate concentration was employed. The titration procedure developed by Linderström-Lang and Sato[1] was adopted.

[1] *Compt. rend. trav. lab. Carlsberg*, No. 17 (1929).

Ten cc. of the edestin solution (containing 40 grams of edestin, 218 cc. of 0.2 N hydrochloric acid and 200 cc. of 0.3 M acetic acid and 0.14 M sodium acetate per liter), and 10 cc. of the malt infusion, both solutions having been attemperated to 37.5°C., were mixed thoroughly in a corked test tube, and 2 cc. of the mixture was pipetted into a flask of 60 cc. capacity, containing 10 cc. of 96 per cent alcohol (this amount of alcohol is sufficient to stop all enzyme action). The alcohol mixture was titrated in the presence of 0.4 cc. of 5 per cent alcoholic thymolphthalein solution with 0.05 N alcoholic potassium hydroxide to a distinct bluish-green color; 20 cc. of hot alcohol was added, and titration was continued to the appearance of the first bluish shade. A similar titration was carried out

TABLE 3.—*Action of malt infusion on edestin at 37.5°C., pH 4.3*

	4 hrs.	*8 hrs.*	*24 hrs.*
Increase in cc. 0.05 N KOH	0.74	1.07	1.90
K =	0.37	0.38	0.39

Control test with boiled malt infusion

	4 hrs.	*8 hrs.*	*24 hrs.*
cc. 0.05 N KOH	4.48	4.50	4.47

Using half and full quantity of malt infusions;
(Temperature 37.5°C; time 24 hours; pH 4.3)

Malt No.	1	2	3	4	5
Half quantity					
Increase cc. 0.05 N KOH	1.55	1.01	0.77	1.13	1.29
K =	0.45	0.30	0.22	0.33	0.37
Full quantity					
Increase cc. 0.05 N KOH	2.30	1.41	1.16	1.64	1.79
K =	0.47	0.29	0.24	0.34	0.37

2 different samples of edestin used; substrates adjusted to the same N content.
(Temperature 37.5°C., time 24 hours; pH 4.3)

Edestin No.	1	2
Moisture	7.66%	5.66%
Nitrogen	16.1%	15.9%

Malt No.	Edestin No.	Increase cc. 0.05 N KOH
1	1	1.55
1	2	1.57
2	1	2.61
2	2	2.62

after incubation of the edestin-malt infusion mixture with 2 drops of toluene for 24 hours at 37.5°C. The increase in titration was taken as a measure of proteolysis. Duplicate titrations on the same infusion agreed within 0.03 cc., and results on the same malt were reproducible within 0.1 cc. The course of reaction appears to follow the Schütz-Arrhenius rule[1]: $X = K\sqrt{et}$, in which X denotes amount of protein digested (cc. 0.05 N KOH), e enzyme concentration, t time of reaction, and K is a con-

[1] Schütz, *Z. physiol. Chem.*, 9, 577 (1885); Arrhenius, Immuno-chemistry, 1907

stant indicating the velocity of action. Table 3 shows data for the action of malt infusion on edestin, some of which is presented graphically in Fig. 3. Control tests with boiled malt infusion produced no change in titration; therefore, no correction was applied to the results obtained. No control experiments were made with malt infusions without the substrate, for, in the opinion of the writer, the total nitrogen digested, that originated from edestin plus the one derived from the malt infusion, is to be considered in these tests. Preliminary experiments with two samples of edestin indicated that they might yield reproducible results when adjusted to the same nitrogen concentration in the substrate; however, the same sample of edestin was used for the 10 malts tested. When combined with

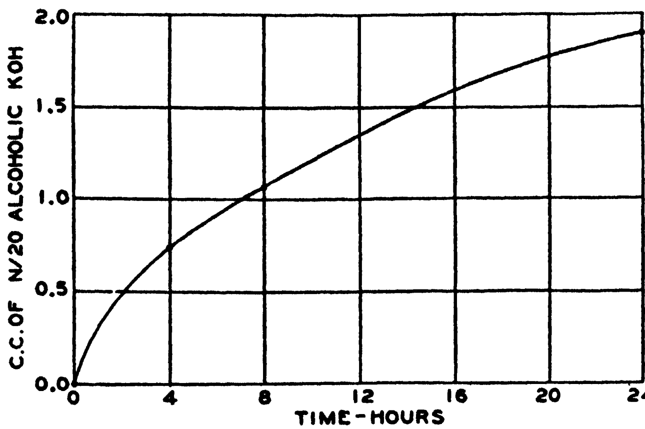

FIG. 3.—ACTION OF MALT INFUSION ON EDESTIN AT 37.5°C., pH 4.3.

a determination of the edestin nitrogen that had been rendered, in the course of digestion, permanently soluble and non-coagulable on boiling, the edestin titration method offers possibilities for measuring the true proteolytic activity of malt infusions. It is being studied further.

Calculation to Proteolytic Activity Units.—The amount of protein digested (cc. 0.05 N KOH), under the conditions specified, by a volume of malt infusion corresponding to a definite quantity of malt, tentatively is suggested as a measure of proteolytic activity. To convert the results to 1, 10, or 100 grams of dry malt, following the Schütz-Arrhenius rule, the titration values might be multiplied by $\sqrt{1/a}$, $\sqrt{10/a}$, or $\sqrt{100/a}$, respectively, where a signifies the amount of dry malt corresponding to the volume of malt infusion used for digestion. In the present tests 10 cc. of malt infusion was mixed with 10 cc. of edestin solution, and 2 cc. of the mixture (containing 1 cc. of malt infusion) was used for titration. For example, malt No. 1, domestic 6-rowed, showed an increase in titration of 2.23 cc. 0.05 N potassium hydroxide for 0.2257 grams, which indicates 47 proteolytic activity units for 100 grams of dry malt.

Gelatin Titration Method

The malt infusions were also allowed to act upon gelatin as a substrate, Willstätter's titration method being used to measure the extent of hydrolysis. This test was undertaken in order to gain information as to whether or not a proportional relationship exists between the proteolytic strengths of a malt infusion: (1) when determined by both the viscometric and titrimetric methods employing the same substrate (gelatin); and (2) when using the same procedure (titration) on such different substrates as are edestin and gelatin.

The same gelatin solution prepared for viscometric measurements was employed in this procedure for testing the 10 samples. The reaction conditions were: 6 per cent substrate concentration, pH 5.0, time 24 hours, and temperature 37.5°C. Eighty grams of the 15 per cent gelatin solution was brought with 20 cc. of Sörenson's citrate buffer of pH 5.0 and sufficient water to 100 cc. Ten cc. of this 12 per cent gelatin solution was thoroughly mixed with 10 cc. of the malt infusion at 37.5°C. From this point on the procedure followed exactly the edestin titration method. The gelatin titration at zero time presented difficulties due to formation of a curd and occlusion of acids, which led to discrepancies in results that required many check determinations. The method could be rendered suitable by introducing a modified titration procedure. The tests also showed that the reaction appears to follow the Schütz-Arrhenius rule (Table 4). There are indications that gelatins of various origin might be brought by proper treatment to yield reproducible results. The units were calculated in the same manner as in the Edestin titration method.

TABLE 4.—*Action of malt infusion on gelatin at 37.5°C., pH 5.0, 24 hours*
Half and full quantity of malt infusions

MALT NO.	1	2	3	4
Half quantity				
Increase cc. 0.05 N KOH	1.18	1.01	0.90	1.27
K=	0.34	0.29	0.27	0.35
Full quantity				
Increase cc. 0.05 N KOH	1.65	1.50	1.11	1.69
K=	0.34	0.31	0.28	0.37

Different gelatins

Malt No.	Gelatin No.	Increase cc. 0.05N KOH
1	1	1.29
1	2	1.39
2	1	1.69
2	2	1.69

Preparation of the Malt Infusions

The samples were finely ground as for extract determination and extracted for 2 hours with 4 times their weight of distilled water at 20°C.;

after filtration through folded filter paper, the solutions were used on the same day for measuring the proteolytic activity. Most of the time the same infusions were used for all three tests; for viscosity measurements they were centrifuged prior to use. From the moisture content of the malt and specific gravity of the solution the amount of malt corresponding to 1 cc. infusion was calculated. For example, malt No. 1, domestic 6-rowed, shows moisture content of 5.4 per cent, and specific gravity of infusion 1.02048, corresponding to 5.2 per cent extract (Plato); the extract yield (dry basis) then is $P \times (M+W)/100-P$, where P=extract (Plato), M=moisture in %, W=amount of water used for extraction of 100 grams of malt; $5.2 \times (400+5.4)/100-5.2=22.3$; $405.4+22.3=427.7$ grams of infusion containing 22.3 grams of extract; $427.7/1.02048=419.1$ cc.; $94.6/419.1=0.2257$ gram of dry malt corresponding to 1 cc. of infusion. The corresponding values for all malts are given in Table 9.

The question as to what temperature could be used for extraction without affecting the proteolytic activity requires further investigation. A few tests indicated that, contrary to the findings by Kolbach and Simon,[1] little or no reduction in activity occurred when the malts were extracted for 3 hours at room temperature or for 1 hour at 40°C. Results of these tests presented in Table 5 illustrate this point.

TABLE 5.—*Comparison of proteolytic activity of malt infusions extracted at different temperatures*

EXTRACTION	VISCOSITY 10% CHANGE	EDESTIN TITRATION 0.05 N KOH	GELATIN TITRATION 0.05 N KOH
	minutes	*cc.*	*cc.*
1 hour at 20°C.	56		
2 hours at 20°C.	58	2.18	1.39
3 hours at 20°C.	58		
1 hour at 40°C.	58	2.19	1.39

All results obtained from the three tests used for measuring the proteolytic activity of malt infusions are presented in Table 9.

Mash Proteolysis

The extent of proteolysis occurring in a laboratory mash was measured by the increase in permanently soluble and formol nitrogen from the cold to the regular mash. In order to eliminate the influence of the total nitrogen content, which varies from malt to malt, the results were expressed in percentage of malt nitrogen. It was thought desirable to make a comparison between these values and the proteolytic activity values of the infusions as obtained by the three methods employed. Table 10 presents this data and, in addition, a comparison of the most important characteristics of the malts tested.

[1] *Loc. cit.*

TABLE 6.—*Physical analysis of malts*

	1 DOMESTIC 6-ROWED	2 DOMESTIC 6-ROWED (MAN-CHURIA)	3 ODER-BRUCKER 6-ROWED	4 WISC. NO. 38 6-ROWED	5 PACIFIC COAST 6-ROWED	6 CALIF. 6-ROWED	7 DOMESTIC 6-ROWED	8 DOMESTIC 2-ROWED	9 FOREIGN 2-ROWED	10 CZECHO-SLOVAKIAN 2-ROWED
Bushel weight (lbs.)	38	36½	35	36	35½	37	38	43½	41¾	41½
1000 Kernel weight (grams)										
as is	29.2	23.4	25.0	24.5	34.6	36.8	23.6	36.0	33.2	34.4
dry basis	27.6	22.6	23.5	22.9	32.9	35.7	21.4	33.5	31.1	32.4
Acrospire growth (%)										
Length of kernel 0–¼	3	1	1	1	1	1	3	1	1	1
¼–½	5	3	1	3	7	1	8	5	8	1
½–¾	6	6	5	3	13	6	7	8	44	19
¾–1	85	90	86	89	78	91	82	86	47	79
over 1	1	0	7	4	1	1	0	0	0	0
Foreign seeds (%)	½	½	¼	few	1	¼	¼	¼	¼	¼
Broken kernels (%)	¼	¼	¼	few	½	¼	few	½	¼	¼
Mold	none	none	none	none	none	none	none	none	none	none
Mealiness (%)										
Mealy	94	95	94	95	94	96	94	97	96	95
Half glassy	4	4	5	4	4	3	5	2	3	4
Glassy	2	1	1	1	2	1	1	1	1	1
Assortment (%)										
on 8/64 inch screen	18.3	2.7	8.1	4.9	26.2	59.6	10.2	36.0	9.3	27.3
on 7/64 inch screen	38.3	14.3	30.8	23.4	34.2	27.7	29.1	31.5	43.8	53.3
on 6½/64 inch screen	31.7	42.5	47.6	51.9	25.9	9.6	44.1	24.6	42.3	18.3
on 5½/64 inch screen	9.9	34.7	11.9	18.0	9.4	2.7	14.6	6.8	4.1	1.1
on 5/64 inch screen	1.3	5.2	1.2	1.5	2.7	0.4	1.8	0.8	0.3	0.0
through 5/64 inch screen	0.5	0.6	0.4	0.3	1.6	0.0	0.2	0.3	0.2	0.0

TABLE 7.—*Chemical analysis of malts: laboratory mash*

	1 DOMESTIC 6-ROWED	2 DOMESTIC 6-ROWED (MAN-CHURIA)	3 ODER-BRUCKER 6-ROWED	4 WISC. NO. 38 6-ROWED	5 PACIFIC COAST 6-ROWED	6 CALIF. 6-ROWED	7 DOMESTIC 6-ROWED	8 DOMESTIC 2-ROWED	9 FOREIGN 2-ROWED	10 CZECHO-SLOVAKIAN 2-ROWED
Moisture (%)	5.4	4.3	6.1	6.4	5.3	5.1	9.5	7.4	6.3	6.4
Extract (Plato)	7.963	7.913	7.913	7.717	7.743	8.075	7.618	8.560	8.328	8.488
Fermentable extract (%)	5.28	4.77	5.18	4.94	4.82	4.94	4.55	5.12	5.21	
Yield of extract (%) as is,	69.7	69.1	69.3	67.4	67.6	70.7	66.8	75.6	73.3	74.8
Conversion (minutes)	under 5	5–7	under 5	under 5	10–12	7–10	5–7	under 5	5–10	5–7
Color of laboratory wort, Lovibond tintometer, half-inch cell, series 52	1.8	1.8	1.8	1.7	1.6	2.3	1.6	1.5	1.9	1.9
Degree of clarity	clear	clear	clear	clear	hazy	hazy	clear	clear	hazy	slight haze
Speed of filtration	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
*p*H of laboratory wort	5.76	5.59	5.93	5.91	5.88	5.85		5.80	5.88	5.98
Soluble nitrogen (mg. in 100 cc.)	98.0	95.0	93.2	79.0	71.0	51.0	80.0	81.5	75.3	87.0
Permanently soluble nitrogen (mg. in 100 cc.)	77.8	88.0	86.2	73.4	68.0	48.0	70.0	74.0	71.1	75.0
Formol nitrogen (mg. in 100 cc.)	23.3	23.3	27.1	21.5	19.0	14.8	30.5	20.4	17.7	21.2
				In 100 grams dry malt						
Yield of Extract (%)	73.7	72.2	73.8	72.0	71.4	74.5	73.8	81.6	78.2	79.9
Fermentable extract (%)	47.4	42.2	46.8	44.7	43.1	44.2	42.8	47.2	47.4	

TABLE 7.—*Continued*

	1 DOMESTIC 6-ROWED	2 DOMESTIC 6-ROWED (MAN-CHURIA)	3 ODER-BRUCKER 6-ROWED	4 WISC. NO. 38 6-ROWED	5 PACIFIC COAST 6-ROWED	6 CALIF. 6-ROWED	7 DOMESTIC 6-ROWED	8 DOMESTIC 2-ROWED	9 FOREIGN 2-ROWED	10 CZECHO-SLOVAKIAN 2-ROWED
Degree of fermentation	64.3	58.4	63.4	62.1	60.4	59.3	58.0	57.8	60.6	
Malt nitrogen (%)	2.45	2.19	2.09	2.05	2.07	1.61	2.35	1.80	1.82	1.76
Protein (N×6.25) (%)	15.3	13.7	13.1	12.8	12.9	9.5	14.7	11.3	11.4	11.0
Soluble nitrogen (%)	0.879	0.840	0.843	0.715	0.635	0.456	0.752	0.751	0.684	0.792
Soluble nitrogen in % of malt nitrogen	35.9	38.4	40.3	34.9	30.7	28.3	32.0	41.7	37.6	45.0
Permanently soluble nitrogen (%)	0.698	0.778	0.779	0.664	0.608	0.429	0.658	0.682	0.646	0.683
Permanently soluble nitrogen in % of malt nitrogen	28.5	35.5	37.3	32.4	29.4	26.7	28.0	37.9	35.5	38.8
Formol nitrogen (%)	0.209	0.206	0.245	0.195	0.170	0.132	0.287	0.188	0.161	0.193
Formol nitrogen in % of malt nitrogen	8.5	9.4	11.7	9.5	8.2	8.2	12.2	10.4	8.9	11.0
Formol nitrogen in % of soluble nitrogen	23.8	24.5	29.1	27.3	26.8	29.0	38.2	25.0	23.5	24.4
Formol nitrogen in % of permanently soluble nitrogen	29.9	26.5	31.5	29.4	28.0	30.8	43.6	27.6	24.9	28.3
Diastatic power: Degrees Lintner	120	107	149	125	99	39	128	82	66	71
Grams maltose produced by 100 grams of dry malt	490	435	608	509	405	161	522	337	271	289

TABLE 8.—*Chemical analysis of malts: cold water mash*

	1 DOMESTIC 6-ROWED	2 DOMESTIC 6-ROWED (MANCHURIA)	3 ODERBRUCKER 6-ROWED	4 WISC. NO. 38 6-ROWED	5 PACIFIC COAST 6-ROWED	6 CALIF. 6-ROWED	7 DOMESTIC 6-ROWED	8 DOMESTIC 2-ROWED	9 FOREIGN 2-ROWED	10 CZECHOSLOVAKIAN 2-ROWED
Extract (Plato)	2.253	2.150	2.393	2.170	1.913	1.693	2.083	2.135	1.903	1.943
Fermentable extract (%)	1.15	1.13	1.14	1.19	1.09	0.91	1.09	1.13	0.98	1.06
Yield of extract, as is	18.6	17.7	19.8	17.9	15.7	13.9	17.2	17.6	15.6	16.0
*p*H of laboratory wort	5.88	5.78	6.33	6.33	6.05	6.15	5.71		6.02	6.00
Soluble nitrogen (mg. in 100 cc.)	71.7	60.0	68.9	57.4	54.0		59.0	59.0	54.3	55.0
Permanently soluble nitrogen (mg. in 100 cc.)	54.9	46.0	51.5	42.6	36.0	45.0	43.0	45.0	38.1	41.0
Formol nitrogen (mg. in 100 cc.)	18.7	15.9	18.7	13.3	12.0	16.0	13.5	15.1	12.2	13.6
			In 100 grams dry malt							
Yield of extract (%)	19.7	18.5	21.1	19.1	16.6	14.6	19.0	19.0	16.6	17.1
Fermentable extract (%)	9.97	9.64	9.96	10.4	9.39	7.80	9.86	9.97	8.49	9.26
Degree of fermentation	50.6	52.1	47.2	54.5	56.6	53.4	51.9	52.5	51.1	54.2
Soluble nitrogen (%)	0.621	0.512	0.602	0.501	0.465		0.533	0.521	0.470	0.480
Soluble nitrogen in % of malt nitrogen	25.4	23.4	28.8	24.4	22.5		22.7	28.9	25.8	27.3
Permanently soluble nitrogen (%)	0.476	0.393	0.450	0.372	0.310	0.386	0.389	0.397	0.330	0.358
Permanently soluble nitrogen in % of malt nitrogen	19.4	17.9	21.5	18.2	15.0	24.0	16.5	22.1	18.1	20.3
Formol nitrogen (%)	0.162	0.136	0.163	0.116	0.103	0.137	0.122	0.133	0.106	0.119
Formol nitrogen in % of malt nitrogen	6.6	6.2	7.8	5.7	5.0	8.5	5.2	7.4	5.8	6.8
Formol nitrogen in % of soluble nitrogen	26.1	26.6	27.1	23.2	22.2		22.9	25.5	22.6	24.8
Formol nitrogen in % of permanently soluble nitrogen	34.0	34.6	36.2	31.2	33.2	35.5	31.4	33.5	32.1	33.2

DISCUSSION

The physical and chemical characteristics of the malts used in this investigation as revealed by their analyses are apparent from Tables 6–8. Of the 10 samples used, seven are of the 6-rowed and three of the 2-rowed type. All 6-rowed are of domestic origin and so also is one of the two-rowed. In general, each sample manifests characteristics that are normal for the type of malt which the sample represents. Five 6-rowed malts belong to the small grain type, with relatively low 1000-kernel weight, high nitrogen content, and high diastatic power; they filter clear and their time of conversion is under 5 and between 5 to 7 minutes. Of these last-mentioned malts, sample No. 3, Oderbrucker, exhibits in the cold mash the highest values for yield of extract, soluble, permanently soluble, and formol nitrogen in percentage of malt nitrogen, which might indicate a pronounced modification of the malt; some of these values are highest for all malts tested, including the 2-rowed varieties. With a few exceptons, the same applies to the laboratory mash of this malt. In addition this sample, of all malts tested, has the highest diastatic power (149°L.), and second highest degree of fermentation in the laboratory mash. On the other hand, malt No. 7, domestic 6-rowed belonging to the same group, shows the lowest figures (for that group) for soluble and permanently soluble nitrogen in percentage of malt nitrogen in both cold and laboratory mash, and for formol nitrogen in percentage of malt nitrogen in the cold mash only; whereas in the laboratory mash the formol nitrogen is the highest for all malts tested. Wisconsin No. 38, of the same group, exhibits

TAPLE 9.—*Proteolytic activity of malts*
(As carried out on their infusions)

	DRY MALT CORRESPONDING TO 1 CC. OF THE INFUSION	VISCOSITY METHOD		EDESTIN TITRATION METHOD		GELATIN TITRATION METHOD	
		10% CHANGE	P. A. UNITS IN 100 G. DRY MALT	CC. 0.05 N ALC. KOH	P. A. UNITS IN 100 G. DRY MALT	CC. 0.05 N ALC. KOH	P. A. UNITS IN 100 G. DRY MALT
	grams	*minutes*					
1. Domestic 6-rowed	0.2257	46	96.3	2.23	47.0	2.01	42.3
2. Domestic 6-rowed (Manchuria)	0.2278	54	81.3	2.42	50.7	1.44	30.2
3. Oderbrucker 6-rowed	0.2236	47	95.1	2.42	51.1	1.42	30.0
4. Wisconsin No. 38, 6-rowed	0.2228	66	68.0	1.88	39.8	0.85	18.0
5. Pacific Coast 6-rowed	0.2254	60	74.0	1.87	39.4	1.13	23.8
6. California 6-rowed	0.2259	130	34.0	1.51	31.8	0.67	14.1
7. Domestic 6-rowed	0.2155	68	68.2	2.09	45.0	1.00	21.5
8. Domestic 2-rowed	0.2205	40	113.4	2.59	55.2	1.61	34.3
9. Foreign 2-rowed	0.2231	60	74.7	2.46	52.0	1.43	30.3
10. Czechoslovakian 2-rowed	0.2228	60	74.8	2.42	51.2	1.94	41.1

TABLE 10.—*Comparison of some important characteristics of malts*

	1 DOMESTIC 6-ROWED	2 DOMESTIC 6-ROWED (MANCHURIA)	3 ODERBRUCKER 6-ROWED	4 WISC. NO. 38 6-ROWED	5 PACIFIC COAST 6-ROWED	6 CALIF. 6-ROWED	7 DOMESTIC 6-ROWED	8 DOMESTIC 2-ROWED	9 FOREIGN 2-ROWED	10 CZECHOSLOVAKIAN 2-ROWED
1000 Kernel weight (grams) dry basis	27.6	22.6	23.5	22.9	32.9	35.7	21.4	33.5	31.1	32.4
Yield of extract, dry basis (%)	73.7	72.2	73.8	72.0	71.4	74.5	73.8	81.6	78.2	79.9
Protein (N×6.25), dry basis (%)	15.3	13.7	13.1	12.8	12.9	9.5	14.7	11.3	11.4	11.0
Diastatic power: Degrees Lintner, dry basis	120	107	149	125	99	39	128	82	66	71
Proteolytic units in 100 g. dry malt										
Viscosity method	96.3	81.3	95.1	68.0	74.0	34.0	68.2	113.4	74.7	74.8
Edestin titr. method	47.0	50.7	51.1	39.8	39.4	31.8	45.0	55.2	52.0	51.2
Gelatin titr. method	42.3	30.2	30.0	18.0	23.8	14.1	21.5	34.3	30.3	41.1
Increase in values from cold to laboratory mash:										
Permanently soluble nitrogen (%)	0.222	0.385	0.329	0.292	0.298	0.043	0.269	0.285	0.316	0.325
Permanently soluble nitrogen in % of malt nitrogen	9.1	17.6	15.7	14.2	14.4	2.7	11.5	15.8	17.4	18.5
Formol nitrogen (%)	0.047	0.070	0.082	0.079	0.067	−0.005	0.165	0.055	0.055	0.074
Formol nitrogen in % of malt nitrogen	1.9	3.2	3.9	3.9	3.2	−0.3	7.0	3.1	3.0	4.2

no abnormal characteristics; only the formol nitrogen in percentage of malt nitrogen is second lowest in that group.

Of interest are the 6-rowed varieties Nos. 5 and 6, both of the Pacific Coast type, of characteristically high 1000-kernel weight, No. 5 of relatively high and No. 6 of low protein content, the latter produced from a type of barley that usually is exported to England. These malts show slow conversion and hazy filtration of the worts; the Californian malt remained hazy even after boiling. Both exhibit in the cold mash the lowest yield of extract and No. 5 the lowest permanently soluble and formol nitrogen in percentage of malt nitrogen, while, surprisingly, No. 6 the highest corresponding values, for all malts under test. Both show in the laboratory mash the lowest figures for soluble nitrogen in percentage of malt nitrogen for all malts examined, No. 6, furthermore, the lowest permanently soluble nitrogen in percentage of malt nitrogen, and both, the lowest formol nitrogen in percentage of malt nitrogen for all varieties tested. In addition, malt No. 6 is unusually low in diastatic power and in all the values representing proteolytic activity and proteolysis in the course of mashing; that is, figures for increase in permanently soluble and formol nitrogen are low, or nil.

The 2-rowed varieties are relatively high in 1000 kernel weight and in extract, and low in protein content and diastatic power. When compared with the imported malts, the domestic 2-rowed sample shows better growth, faster conversion, and clearer filtration of wort; in the cold mash it exhibits higher values for soluble, permanently soluble, and formol nitrogen in percentage of malt nitrogen, which points towards more marked modification. When compared with the 6-rowed samples, the two-rowed malts, in general, manifest in the laboratory mash higher values for permanently soluble and (partly also) for soluble and formol nitrogen in percentage of malt nitrogen.

No relationship could be observed between the diastatic power values and increase in degree of fermentation from cold to laboratory worts. It may be noted that the hydrogen-ion concentration of the worts shows considerable variation from malt to malt; there is always a drop in the *p*H value from cold to laboratory mash.

The measurement of the proteolytic activity of the malt infusions by the three methods described above was carried out under conditions which apparently exclude the action of peptidases. These enzymes found in green malt are known to become considerably weakened during the kilning process; in addition they are very unstable and show slight activity at the hydrogen-ion concentrations at which the tests were conducted. It may thus be assumed that under the conditions specified the action of the proteinase only was determined. It is also doubtful that peptidases develop any marked activity in a laboratory or brewery mash.

The proteolytic activity units as revealed by the three methods differ

from sample to sample and do not show parallel relationships or definite trends. It is quite possible that the proteinase of malt infusions obtained from different malts has several components, which in their activity may vary from malt to malt. In one malt infusion one activity may predominate over the other, and vice versa. Another explanation may be found in Willstätter's conception of enzyme composition and action. According to this investigator, enzymes are composed of a colloidal protein carrier and of one or more active groups. While the colloidal carrier is responsible for the catalytic activity and the stability of the active groups, to the latter is due the specificity of enzymic action. Even small changes in the chemical nature of the active groups leads to great changes in enzymic specificity. However, changes in the composition of the colloidal carrier causes not a qualitative but a quantitative change in activity; that is, the specificity is the same, but the rate of action may either increase or decrease. In addition, many enzymes, according to Willstätter, may occur in soluble and insoluble form, depending upon the solubility of the protein carrier with which they are combined. They are called lyo-enzymes and desmo-enzymes; by autolysis the desmo-enzymes are transformed into lyo-enzymes. It may be assumed that several protein carriers are associated with the various components of the malt proteinase—in one malt infusion more of a particular colloidal carrier goes into solution than in the other. This may account for the differences in proteolytic activity of the infusions as estimated by the three methods.

It is of interest to note that in general the 2-rowed malts show a higher proteolytic activity, as demonstrated by all methods, than the 6-rowed varieties, especially as shown by the edestin and gelatin titrations, and by the increase in permanently soluble nitrogen based on percentage of total nitrogen of malt. The domestic 2-rowed shows the highest values by the viscometric and edestin methods. The proteolytic activity as indicated by the results presented for the methods used (with exception of No. 6 California malt) definitely does not run parallel to the diastatic power. The 2-rowed malts Nos. 8, 9, and 10, with diastatic powers varying from 66 to 82° Lintner, present the highest average values for proteolytic power. Many questions present themselves as a result of these figures. Does the development of proteolytic activity under certain conditions of malting proceed more rapidly than that of diastatic enzymes, or is the diastase more sensitive to the kilning process than the proteolytic power? The question of activators, colloidal carriers, specificity of malt proteinase, and other influences also present unsolved problems in this field which require further study.

When attempting to formulate a relationship between the proteolytic units of the infusions, as developed through tests with controlled substrates, and the extent of proteolysis as shown by increase in permanently soluble and formol nitrogen, it must be noted that the nitrogenous sub-

strates supplied by the malt mash present an uncontrolled condition which may vary largely from malt to malt. Clarification of this problem and an advanced understanding of the action and activity of proteolytic enzymes of malt must await the outcome of further and far more extensive studies on malt proteins and their degradation products, and on other nitrogenous substances.

SUMMARY

An attempt was made to apply three different methods for measuring the proteolytic activity of malt infusions derived from 10 malts of various origins, characterized by their physical and chemical analyses.

One method was based on changes in viscosity of a gelatin solution effected by a proteolytic enzyme. In this case it was found possible to define the activity of malt proteinase in terms of the time required to cause a definite change in the initial viscosity of the substrate. The second method was that of measuring the extent of proteolysis on edestin by the Willstätter titration in alcoholic solution. The third method was similar to the second with the exception that edestin was substituted by gelatin as a substrate.

Tentative units were suggested for the proteolytic activity as determined by the three methods. In addition, the extent of proteolysis occurring in a laboratory mash was measured by the increase (from a cold to a regular mash) in permanently soluble and formol nitrogen, expressed in percentage of malt nitrogen.

The results obtained by the three methods for the proteolytic activity of malt infusions differ from sample to sample, and show no definite trends or parallel relationships. The same applies to the proteolytic activity as secured by the three methods when compared to the extent of proteolysis effected in a laboratory mash. A tentative explanation for these discrepancies is offered.

Between the proteolytic activity and the Lintner value of the malts tested no proportional relationship was found to exist.

The author wishes to acknowledge the constant encouragement given to this work by Robert Schwarz, director, and the assistance of Louis Laufer and Henry Ziliotto, members of the staff of Schwarz Laboratories Inc., in developing part of the data presented in this paper.

BOOK REVIEWS

A Textbook of Bacteriology and Its Application. Revised Edition. By CURTIS M. HILLIARD, Professor of Biology and Health, Simmons College. Ginn & Co., Boston, 1936. Price $3.50.

The original edition has been extensively revised, and in the new edition sections on "Life Cycles of Bacteria," "How Bacteria Cause Disease," "Bacteriophage" and "The Acute Food-infection Group" have been added. The writer was somewhat surprised to find, after reading the first chapter, which is devoted to the history of bacteriology (supplemented by a chronological listing of important events in Appendix A), that Chapter II discusses the molds, Chapter III the yeasts, and Chapter IV then returns to the bacteria. The classification of bacteria presented in Bergey's "Manual of Determinative Bacteriology," Fourth Edition, 1934, is followed throughout this textbook (Chapter V). Chapter VII presents a well-balanced discussion of bacterial nutrition and respiration, while in the following chapter the relationship between the reactions of bacteria to physical factors of environment, such as temperature, light, electricity, and osmotic pressure, is shown. On page 108 (Chapter VIII) the thermogenic powers of bacteria are discussed, with the statement that the claim that heat evolved by fermentation is sufficient to cause spontaneous combustion is a fallacy. Information on this point published recently reveals that bacteria and other microorganisms are not claimed to produce fire, but rather to produce some heat and certain chemical compounds, which when oxidized, will lead to fire. This is not a fallacy, but appears highly probable. Those who have studied the spontaneous heating of agricultural products will not agree that "self-heating of hay, tobacco, and moist grains is of a purely chemical nature and is brought about by the plant cells." The following Chapter, IX, titled "Bacteria and the Chemical Environment," is really a discussion of disinfection. The types of chemical environment concerned with the vast majority of bacteria are presented in the chapter on "Nutrition and Respiration of the Bacteria." The text also contains sections on methods of studying bacteria, enzymes, fermentation of carbon compounds, fermentation of nitrogen compounds, water, milk, and food preservation. This latter section is, necessarily, an abbreviated discussion of the vast amount of material available on canning, preserving, dehydration, refrigeration, etc. Those familiar with the refrigeration of poultry will disagree with the statement (p. 199) that "The study of the relative keeping qualities of drawn (eviscerated) and undrawn poultry has demonstrated in a striking way the advantage of leaving the birds undressed until just before use." Data are reproduced from Bureau of Chemistry Circular 70 (which was published in 1911) in support of this statement. While unfortunately there are very few recent data on this point, and although the great majority of poultry held in cold storage is not drawn before storage, nevertheless the real reason is a lack of scientific study and development of up-to-date methods, rather than because of any weakness inherent in the process of drawing the birds. Modern methods of handling poultry and the much improved conditions for freezing would quite certainly invalidate the statement in the text.

On the whole the textbook accurately presents bacteriology in its many applied fields other than as related to disease, and it is recommended to all who are interested in the diverse applications of microorganisms to life.—LAWRENCE H. JAMES.

Fifty Years of Field Experiments at the Woburn Experimental Station. By SIR E. JOHN RUSSELL and J. A. VOELCKER, with a statistical report by W. G. COCHRAN. 392 pp. Longmans, Green & Company, London, New York, Toronto. Price $7.50.

The Woburn Experimental Station was founded in 1876 by the Duke of Bedford and the Royal Agricultural Society of England at a time when the Rothamsted

Experimental Station, although more than 30 years old, was the only one in existence in Great Britain and the private property of the Sir John Lawes. The conduct of the experiments have been in the hands of Dr. J. A. Voelcker since 1884.

This book is intended to give a summary of the important results during 50 years of the experiments, with the exception of the pot experiments. The book is in four parts. Part I, an account by Dr. Voelcker, summarizes the experiments on the residual manurial values of cake and corn fed to animals, on the continuous growth of wheat and of barley on the same land, on green-manuring and green-cropping, on grass and other fodder crops, and on the feeding tests made. Part II is a statistical examination of the results by W. G. Cochran. Part III is a discussion of the results and their bearing on agricultural science and practice by Sir E. John Russell. Part IV contains physical and chemical analyses of the soils of the Woburn plats by Dr. E. M. Crowther. The appendix contains detailed results, such as those on date of sowing, varieties of seed, manures applied, yields of barley and wheat, temperature, and rainfall. Among the valuable results of this work to which attention is called is the general need for nitrogenous fertilizer, whether the soil be light or heavy, the need of lime when sulfate of ammonia is used frequently, the fact that green manuring is not an entirely trustworthy practice, and the serious losses from farmyard manure. The results of the work are clearly brought out as well as the defects afterwards found to have been made in the planning of pioneering experiments. The book is a clear and valuable presentation of the results of the Woburn experiments. It is readable, and the material is well presented.—G. S. FRAPS.

The Scientific Principles of Plant Protection. With Special Reference to Chemical Control. Second edition. By HUBERT MARTIN. Edward Arnold and Company, London, 1936. Price $8.00.

This book is a study of the factors that affect plant protection. It deals first with natural controls, including plant resistance, the effects of soil, climatic and cultural methods, and biological controls of insects and plant diseases. Then, the main portion of the book deals with insecticides and fungicides, and with their adjuncts, such as spreaders, stickers, dispersing agents, and emulsifiers. Some attention is also given to weed killers, soil treatment, relation of toxic action to chemical composition, and the elimination of centers of infection and carriers.

The book is written largely from a chemical standpoint, the chemistry of insecticides and fungicides being well presented, and much information is also given on the physical and biological factors affecting their application. This edition has been brought up-to-date, and it should be especially useful to the research chemist who is investigating insecticides and fungicides or developing new ones, and should also be of value to the entomologist or plant pathologist in suggesting possible treatments for the insects or diseases that he may desire to control.

There are over 1200 citations to the literature, with American articles well represented and many of them quite recent. The indexing is very thorough.—E. L. GRIFFIN.

George Leslie Bidwell, 1880–1937

GEORGE LESLIE BIDWELL

At 12:35 on Saturday morning, February 20, 1937, George Leslie Bidwell went on what has been called "The Great Adventure." As sometimes happens to those more fortunate victims of cerebral hemorrhage, death came suddenly and with no apparent suffering or premonitory symptoms.

The last night of his life was so typical of a many-sided character—and every side lovable—that it is worthy of brief descripton. In the early part of the evening there had been one of those little "family parties" that involve dining out, and that give a much needed respite to the meal planner. Somewhat later a young man, who had learned feeding stuffs control under Mr. Bidwell's tutelage, came in for some helpful advice on Association and State control problems. Still later on his fine stamp collection claimed attention, and it was while he was in that pleasant glow, which perhaps only a philatelist can fully appreciate, that the end came.

Mr. Bidwell was born August 31, 1880, at Goffstown, among the scattered mountains and clear lakes of Southern New Hampshire. His early years, up to the time he attended high school near Boston, were spent on a farm, and it was perhaps here that he got his first inspiration to make the chemistry of grains and stock feeds his specialty. At any rate he profited from that best of teachers, in his generation at least, the New England farm. His parents were people of innate culture and education, and he came honestly by his love of good books, good music, and the other fine things of life. His father possessed the ability, energy, and vision to steadily improve his economic status so as to keep pace with the growing demands of what would now be regarded as a large family of children—five boys and one girl. George, the eldest child, graduated from Tufts College in 1905, with a B.S. in chemistry. For a year he was employed in the laboratories of the B. S. Sturtevant Company of Hyde Park, Massachusetts, but his taste for the intangible but lasting satisfactions of life soon led him away from commercial pursuits. He was on the chemistry faculty of the Rhode Island State College for fifteen months, and during this period married Miss Sarah Haynes, who survives him.

Those were the days of the passage of the Food and Drugs Act, when Harvey W. Wiley was a flaming meteor across the skies in the early dawn of Federal regulatory work. On December 8, 1907, George Bidwell became one of "Wiley's boys," and one of those fortunate few whom we now affectionately call "The Old Guard." He started in the old Miscellaneous Laboratory under Doctor J. K. Haywood, and it was not long before his intimate knowledge of feeding stuffs came to the fore. He was made Chief of the Cattle Feed Laboratory in 1913. In 1927, when the regulatory functions of the old Bureau of Chemistry were placed in the newly created Food and Drug Administration, the feeding stuffs work was transferred to the latter Bureau with Bidwell in charge. Shortly afterwards cereal food products were added to his responsibilities, and he was given the full rank of chemist.

His standing in his chosen field was such that only the merest mention of his services and publications is called for.

In the Association of Official Agricultural Chemists he was Referee on Feeds and Feeding Stuffs in 1916, 1921, and 1922, and Referee on Baking Powder in 1930 and 1936; on the Auditing Committee in 1924, on Subcommittee A in 1933–4–5–6–7, and on the recently formed Com-

mittee on Moisture in 1936–7. For years he was the Patron Saint of the Association of American Feed Control Officials, serving on many of its important committees. He was President in 1925, and Chairman of its Executive Committee continuously from 1928 to the day of his death. He was also a member for many years of the subcommittee of the Federal Specifications Board dealing with the subject matter "Feeds and Forage."

His publications are too well known to enumerate. There is a group dealing with the composition and suitability for use of a wide range of stock feeds; another with the methods of feed analysis such as crude fiber, starch, sugars, and moisture; and a third group (perhaps the birthplace of his later interest in statistical mathematics) that wrestles with the old bugaboos "What is an adequate sample?" and "What is analytical error?"

When it comes to speaking of the more personal aspects of Bidwell's character and gifts, those who knew him best will realize the difficulty of speaking with restraint, for it is rare, indeed, that one finds the combination of kindness, tolerance, and wisdom that he possessed. He was so utterly free from the slightest disposition to be self-seeking and so ready to do others a good turn at the expense of his own time and convenience, that it seems as if the expression "let George do it" must have been originally applied to him!

Perhaps one of the best measures of a man's mental and spiritual stature is the range and character of his interests. Mr. Bidwell had followed radio engineering from its very inception, and had kept thoroughly up-to-date in this complex and rapidly changing field. He had made himself an expert in statistical mathematics, and was the sole refuge of the Administration when problems of sampling first began to manifest themselves in spray residue, net weight, and other regulatory operations. His intimate knowledge of engineering materials and laboratory equipment made him of invaluable service in planning the new laboratories of the Administration. His advice was always sought in the purchase of new apparatus, especially of the complicated and expensive type where one can not afford to purchase a "white elephant." He was skilled in the use of tools and the handling of all sorts of materials, whether wood, metal, or even glass. There is today in his home a partially ground set of lenses for a telescope. His interest in stamp collecting has already been mentioned. Like most wise and contemplative spirits from Isaak Walton on he was extremely fond of fishing and would go on the slightest provocation. Another of his enthusiasms was the Masonic Order, the high ideals and the imposing ritual of which, to him as to many, possessed almost a religious import.

All these are but the surface indications of a richly furnished mind. Time and again his close associates have been astonished at some little remark which would reveal an unsuspected depth of knowledge of classical music, of poetry, or even (and this is no imaginary illustration) of the Aztec civilization!

In each of the many circles of Mr. Bidwell's interests and enthusiasms there were large numbers of fellow enthusiasts and friends, to many of whom he had done more than one good turn in the quiet, almost secretive, way that was so characteristic of him. In all of these circles there is now that bewildering sense of loss—and almost of waste—which death brings. But there is too the feeling of gratitude, and almost joy, that such lovable souls can exist in a weary world, and that we were fortunate enough to come within their orbit.

W. B. White

TUESDAY—MORNING SESSION

REPORT ON PLANTS

By O. B. WINTER (Michigan Agricultural Experiment Station, East Lansing, Mich.), *Referee*

The work on methods for the analysis of plant materials appears to show a period of relaxation after the strenuous year of 1935, when plans were being made for the publication of the 4th edition of *Methods of Analysis*. Extensive work was planned for this year, but much of it was not carried to completion. Hence, most of the associate referees are not making formal reports, even though some progress has been made.

Very little collaborative work was done this year, and there is nothing to report along that line. It may be of interest and value, however, to make some statements concerning the practicability of routine work on some of the methods that have been used in the referee's laboratory and that are given in the chapter on plants in the new *Methods of Analysis.*

IRON

The titration method for the determination of iron, p. 122, is being used almost entirely in place of the colorimetric method, which was used almost exclusively for several years. The titration method is simple and rapid, and the results by the two methods agree very closely.

SODIUM AND POTASSIUM

A number of sodium and potassium determinations were made on plant materials. The most practical procedure found was to weigh 4 grams of material, ash according to directions, dissolve, and make to a given volume. The official rapid method for potassium only, p. 126, was used for determining the potassium in an aliquot part of the solution. The tentative uranyl acetate method for sodium only, p. 126, was used for the determination of sodium in a separate aliquot part. These methods are very satisfactory. They are more rapid and more easily manipulated than the older official methods, in which the total amount of sodium and potassium is determined as the chloride, the potassium as the platinic chloride, and the sodium is calculated by difference. Collaborative work should be done on these methods with the view to making them official and deleting the older methods.

The Referee's attention has been called to the fact that the procedure used by Hicks[1] for the determination of potassium is being used in some laboratories. In this procedure the solution is prepared in the usual way and acidified, platinic chloride is added, and the solution is evaporated. The potassium platinic chloride is washed with 80 per cent alcohol, dissolved in hot water, filtered, acidified, and reduced by the addition of

[1] *Ind. Eng. Chem.*, 5, 650 (1913).

magnesium ribbon. The advantage of the method is that the common elements do not interfere with the determination. The method should be studied and compared with the other methods.

COPPER

The Associate Referee on Copper in Foods (Coulson) made an extensive study of the Haddock-Evers method[1] for the determination of copper as modified by Butler[2] for the determination of copper in plants. This method is much shorter and appears to be as accurate as the hydrogen sulfide methods.

LEAD

Several methods have been proposed for the determination of minute quantities of lead. These should be evaluated for their applicability to plant materials.

IODINE

Since the Referee's laboratory does not possess a muffle furnace suitable for burning samples for the determination of iodine, the von Kolnitz-Remming enclosed torch was used. This low priced piece of apparatus was found to be very satisfactory. Undoubtedly this procedure should become an alternative method for ashing the samples for making iodine determinations.

FLUORINE

Work done in the Referee's laboratory on the determination of fluorine in plants was a continuation of the work of last year—a study (1) of the colorimetric method mentioned in last year's report, *This Journal*, **19**, 362 (1935), (2) of alizarine as a dye to form a lake with thorium nitrate, and (3) of different fixatives for ashing the samples. No important progress was made with the colorimetric method. Higher recovery results were obtained with metallic copper and precipitated calcium carbonate than with any other fixatives. It was also noted that the samples burned completely at a lower temperature when these fixatives were used.

NEW PROBLEMS

Two new problems in the analysis of plants have developed during the past year. These show the need for A.O.A.C. methods for the determination of hydrocyanic acid in plants and for the determination of inulin in such materials as dahlia roots, artichokes, chicory, etc. These methods should be studied as soon as an associate referee can be found to carry on this work.

RECOMMENDATIONS[3]

It is recommended—

(1) That the following last year's recommendations be carried over:

[1] *Analyst*, **57**, 495 (1932).
[2] *This Journal*, **19**, 360 (1935).
[3] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 52 (1937).

(a) that the titration method for the determination of iron be compared with the colorimetric method and studied collaboratively with the view to adopting it as official.
(b) that collaborative work be done on the perchlorate method for the determination of sodium and potassium, and on the uranyl acetate method for the determination of sodium with the view to adopting these methods as official.
(c) that studies on forms of nitrogen, carbohydrates, and less common elements in plants be continued by the associate referees.
(d) that studies of methods for the determination of copper, lead, and fluorine in plants be continued.

(2) That the recommendations made by the Associate Referee on Chlorine in Plants be adopted.

(3) That the Hicks method for the determination of potassium referred to in this report be studied, and that an associate referee be appointed for carrying on all the work on sodium and potassium mentioned in this report.

(4) That associate referees be appointed to study methods for the determination of hydrocyanic acid in plants and for the determination of inulin.

No report on less common metals in plants was given by the associate referee.

REPORT ON TOTAL CHLORINE IN PLANTS

By HERBERT L. WILKINS (Bureau of Plant Industry, Division of Forage Crops and Diseases, National Agricultural Research Center, Beltsville, Md.), *Associate Referee*

Studies of the preparation and use of an aqueous iodine solution in determinations of the total chlorine in plants and of the applicability of this tentative method to various materials were recommended in the previous report, *This Journal*, **19**, 366 (1936). Such studies were made this year, and the results of analyses of certain materials by the tentative method, *Methods of Analysis, A.O.A.C.*, 1935, or with slight modifications are listed in Table 1. The nature of the material and experience in applying the method to it will usually indicate necessary changes. For example, if after the usual period of digestion, filtration is slow (as with kelp), a longer digestion period may be provided. Or the sample may be digested as usual and allowed to stand overnight, then re-heated before it is diluted and filtered. An extended digestion should be protected from strong light. Samples that contain much oil or fat (*e.g.*, rice polishings)

require the use of a hot wash solution. More oxalic acid, or other substances that react with potassium permanganate, may be formed in one case than in another, so that for the second application the reagent should be added in small quantities until the color fades slowly, regardless of the amount used at the beginning.

In an effort to save time, some of the Kjeldahl digestions were made over extra hot heaters, and very erratic, incomplete recovery of the chlorine resulted. When moderate heat is used and the digest is completely cooled, before dilution, the error is greatly reduced, but it is not eliminated entirely. The cause of this behavior was suspected to be sulfurous acid, or its salts, since in an acid medium either will form iodides from free iodine. That this was the interfering substance is shown by the fact that when the diluted digest, with or without added sulfurous acid, was boiled for 5 minutes before being cooled and titrated with potassium iodide, correct results were obtained on both blanks and known samples.

In some instances it was found necessary to discard more than the first acidified aqueous solution as directed under Reagent (f). The sulfuric acid used in the preparation and testing of this reagent should be freed of sulfurous acid because the ordinary C.P. reagent sometimes contains enough of this contaminant to give the blue color with starch and iodine.

A solution of iodine in benzene (C_6H_6) of such strength that one or two drops are enough for a titration appears to be the best and most convenient method of adding iodine to the reaction mixture that has been used so far. It was tried so recently that only a few tests could be made.

TABLE 1.—*Chlorine determined by the tentative method*

SAMPLES	CHLORINE FOUND		REMARKS
	per cent		
Sucrose, J. T. Baker's C.P.	0.00	0.00	Unmodified method
Grass, 1935 collaborative sample	0.63	0.62	Unmodified method
Dried sugar beets*	0.06	0.06	Unmodified method
White clover	0.71	0.71	Unmodified method
Dried kelp†	13.66	13.70	Digest stood overnight
Hickory nut shells	0.01	—	Digest stood overnight
Hickory nut meats	0.01	—	Hot wash solution used
Rice polishings‡	0.04	0.04	Hot wash solution used
Orange pulp meal‡	0.04	0.04	Method unmodified
Ground whole oats‡	0.09	0.08	Method unmodified
Hemp seed‡	0.04	0.04	Hot wash solution used
Soybean oil cake meal‡	0.01	0.01	Method unmodified
Dried yeast‡	0.47	0.46	Hot, strongly acid wash solution used on one determination only

* Sample furnished by O. B. Winter, Michigan Experiment Station.
† Sample furnished by H. A. Halvorson, Minnesota Experiment Station, a portion of his 1933 collaborative iodine sample No. 1.
‡ Sample furnished by H. W. Titus, Bureau of Animal Industry.

RECOMMENDATIONS[1]

It is recommended—

(1) That study of reagents for the introduction of free iodine be continued.

(2) That the tentative method be studied collaboratively, if possible.

(3) That the Associate Referee be advised at once when any one encounters difficulty with the method.

(4) That under Reagent **38 (e)**, p. 132, the words "boil for 5–10 min.," be inserted after the words "to 1 liter of water."

(5) That the last two sentences in the description of Reagent **38 (f)**, p. 132, be deleted and the following inserted, "Decant and test the soln by adding 25 cc. of it to 5 cc. of Reagent **(d)** and 25 cc. of Reagent **(e)**. No more than a faint blue should appear after the soln has stood 5 min. If necessary, repeatedly apply fresh portions of Reagent **(e)** to the residue of iodine crystals."

(6) That the sentence, 2nd line from bottom of p. 132, "When the digest is cool, add 75 cc. of H_2O and cool to room temp.," be revised to read, "When the digest is cool, add 175 cc. of H_2O, boil 5–10 min., and cool to room temp."

No report on carbohydrates in plants was given by the associate referee.

No report on forms of nitrogen was given by the associate referee.

No report on sodium and potassium was given by the associate referee. See report of the Referee on Plants.

No report on lignin was given by the referee.

No report on enzymes was given by the referee.

No report on pepsin was given by the associate referee.

No report on papain was given by the associate referee.

No report on waters, brine and salt was given by the referee.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 52 (1937).

REPORT ON MINERAL SALTS AND EFFERVESCENT SALTS

By A. E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on salts comprised the collaborative study of sampling and moisture determinations described previously in *This Journal*, **19**, 376 (1936), and the application of the method to the examination of effervescent salts.

A sample of effervescent salts containing sodium bicarbonate, sucrose, citric acid, sodium citrate, and small quantities of sodium chloride and sodium sulfate was prepared and submitted to two collaborators. The results obtained follow:

COLLABORATOR*	SAMPLE	TOTAL DRYING TIME	MOISTURE
	grams	*hours*	*per cent*
1	0.6331	1.5	2.67
	0.7569	1.5	2.54
	0.5700	2.5	2.70
	0.7926	0.5	2.90
2	0.8190	1.5	1.92
	0.7494	1.5	1.99
	0.3368	1.5	1.93
	0.7517	1.5	1.94

* Collaborator No 1 dried samples in an Abderhalden drier und vacuum, Collaborator No. 2 in an Abderhalden drier under a slow current of air (about 65 bubbles per minute) dried by calcium chloride and sulfuric acid.

Experiments carried out by the Associate Referee in which effervescent salts, after being dried, were cooled for 0.5 hour in glass-stoppered weighing bottles, in a desiccator over calcium chloride, showed conclusively that even under these conditions the salts will increase in weight about 1 mg. Therefore it would appear reasonable for the analyst to consider that the sample has reached so-called constant weight when two successive weighings do not vary more than 1 mg.

Several samples of effervescent salt were placed in glass-stoppered weighing bottles in a desiccator over calcium chloride and allowed to remain 36 hours. The samples lost from 1 to 9 mg. in weight, which again indicates that the ordinary glass-stoppered weighing bottles do not prevent change in weight in this type of product. Some of the samples showed a tendency to increase in weight after successive heatings, and in most of such instances there was a darkening of the sample.

The results show that the greater portion of moisture is given off in 1.5–2 hours of heating and that then the weight becomes practically constant.

Change in weight on continued heating may be ascribed to chemical changes in the composition of the material, since decomposition probably takes place before drying is complete.

It has been noted that while a number of effervescent salts are described in U.S.P. XI and in National Formulary VI, an assay for only one of these (Compound Effervescent Powder, Seidlitz Powder) is required.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be conducted on the present tentative procedure for the determination of moisture in effervescent and mineral salts.

(2) That methods of analysis for effervescent potassium bromide with caffeine and similar preparations be studied.

The paper, entitled "Detection of Decomposition Products in Butter and Cream," presented by J. O. Clarke, will be found on page 475 of this number of *This Journal.*

The paper, entitled "Development of Methods for the Estimation of Mold in Cream or Butter," presented by J. D. Wildman, was published in *This Journal,* **20,** 93 (1937).

No report on dairy products was given by the referee.

No report on butter—preparation of sample and fat—was given by the associate referee.

REPORT ON CHEESE

By CARL B. STONE (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

The methods submitted by the referee in 1934 for the determination of ash and salt in cheese were made tentative by the Association at the 1935 meeting, *Methods of Analysis, A.O.A.C.,* 1935, 291. In order to recheck these methods, a sample of cheese was prepared under the supervision of H. W. Gregory, Department of Dairy Husbandry, Purdue University, Lafayette, Ind. It was thoroughly mixed by grinding in a meat chopper and a half-pint jar filled with the ground cheese and copies of the ash and total chloride methods, as reported in *This Journal,* **18,** 401 (1935), were sent to each collaborator.

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[1]For report of Subcommittee D and action of the Association, see *This Journal,* **20,** 63 (1937).

The following results were obtained for total ash, the temperature not exceeding 550°C., and for total chloride:

	ASH	TOTAL CHLORIDE AS NaCl
	per cent	*per cent*
S. M. Starke, Jr.	3.42	1.42
St. Louis	3.42	1.40
Jonas Carol	3.42	1.39
Cincinnati	3.42	1.39
Harley G. Underwood	3.37	1.38
Cincinnati	3.36	1.40
Oro S. Keener	3.45	1.37
Cincinnati	3.46	1.41
		1.45
Franklin J. McNall	3.39	1.42
Chicago	3.42	1.39
Edward O. Haenni*	3.49	1.43
Washington	3.47	1.42
	3.42	
J. L. Perlman	3.44	1.42
Albany	3.41	1.44
	3.42	1.41
	3.41	
	3.40	
	3.39	
Burton Jordan	3.43	1.42
Vermillion	3.40	1.43
	3.44	1.41
	3.46	1.46
	3.45	1.48
Waldo Johnson	3.38	1.38
St. Paul	3.38	1.37
	3.38	1.37
	3.37	1.38
	3.38	
	3.38	
	3.38	
George Dysterheft	3.39	1.38
St. Paul	3.39	1.39
	3.41	1.38
Carl B. Stone	3.42	1.39
	3.42	1.39
		1.41
Average (11) subs	3.41	1.41

* Sample was in very bad condition, but the Referee requested that the work be completed on the sample as received.

None of the collaborators submitting results offered any criticism of the ash method, and the uniform results indicate that the method is workable.

The total salt figures reported by the collaborators show conclusively that the method is workable and accurate within narrow limits. Several of the collaborators regard the method as rapid and accurate. One of the general comments was that the small amount of fat that is present in the mixture had a tendency to solidify in the neck of the 200 cc. graduate flask when the sample was diluted. In order to eliminate this difficulty a few minor changes were made in the wording of the method. The method as written for final approval follows:

TOTAL CHLORIDES

Weigh accurately approximately 3 grams of cheese into a 300 cc. Erlenmeyer flask and add 25 cc. of 0.1 *N* $AgNO_3$, which is more than enough to combine with all the Cl. Add 10 cc. of halogen-free HNO_3 and 50 cc. of water, and boil. As the solution boils, add approximately 15 cc. of 5% $KMnO_4$ solution in 5 cc. portions. (The solution becomes yellowish and clear.) Cool, and filter into a 200 cc. graduated flask, washing the filter paper thoroughly with water at approximately 20°C., and make to volume. Titrate the excess $AgNO_3$ in 100 cc. of the solution with 0.1 *N* KCNS, using 2 cc. of a saturated solution of ferric alum as indicator. Run a blank on the reagents used, following the same procedure, except to add sugar to destroy the excess permanganate. Calculate the Cl found to NaCl and report as such.

The results secured by the collaborators for ash and total chlorides indicate that these methods are satisfactory.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method for the determination of total ash in cheese be made official (first action).

(2) That the tentative method for the determination of total chlorides in cheese as revised in this report be made official (first action).

REPORT ON MALTED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year it was recommended that the method proposed by the Associate Referee, *This Journal*, **18**, 454 (1935), for the determination of fat and Reichert-Meissl value be studied with a view to its adoption as tentative.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 59 (1937).

The proposed method was used by both the analysts making the original determinations and the Associate Referee, and the Associate Referee checked the fat and R-M values on some commercial samples of chocolate-flavored malted milks. It was found almost invariably that the results for fat obtained by the Associate Referee were higher than those obtained by the other analysts. Possibly the filter cel cake was not sufficiently dry to obtain complete extraction of the fat with petroleum ether. A check determination, in which precaution was taken to dry the filter cel cake to a fine powder, increased the return of fat appreciably, and in most cases checked the value found by the Associate Referee.

Next year samples of malted milks will be sent to collaborators, and a caution will be inserted in the method in regard to drying the filter cel cake to a fine powder prior to extraction with petroleum ether.

RECOMMENDATIONS[1]

It is recommended—

(1) That the determination of fat and Reichert-Meissl value by the method proposed be further studied collaboratively.

(2) That last year's recommendation for the determination of casein and for the study of the type of mounting suggested by Ballard be carried over.

REPORT ON DRIED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

At the meeting last year mention was made of a colorimetric method being developed by the Associate Referee for the determination of lactic acid in milk and milk products. At that time it was hoped that it might be possible to submit the method to collaborators, but this was found to be impossible.

The procedure is based upon a color reaction with ferric chloride, and the color is read in a photometric colorimeter. Varying quantities of lactic acid were added to dried skim milk of a good quality, and it was found possible to return practically 100 per cent of the added acid. No trouble was experienced in applying the method to a wide variety of milk products. On a majority of the commercial and authentic milk products analyzed the lactic acid content ranged from 1 to 7 mg. per 100 grams. The method is sensitive to about 10 p.p.m.

It is expected that a complete report on the method can be given later (see *This Journal*, 20, 130 (1937)).

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 59 (1937).

It is recommended[1] that methods for the determination of lactic acid be studied collaboratively, and also that methods for the detection of neutralizers be studied.

No report on milk proteins was given by the associate referee.

REPORT ON LACTOSE IN MILK

CLARIFICATION OF MILK WITH ACID MERCURIC NITRATE AND PHOSPHOTUNGSTIC ACID PRECEDING THE OPTICAL DETERMINATION OF LACTOSE

By E. R. GARRISON (University of Missouri, Columbia, Mo.), *Associate Referee*

The method recommended by this Association for the clarification of milk with acid mercuric nitrate preceding the saccharimetric determination of lactose, *Methods of Analysis, A.O.A.C.*, 1935, 266, is essentially the same as that proposed years ago by Wiley.[2] By this method twice the normal weight of milk (65.80 grams) is precipitated with 1 cc. of acid mercuric nitrate solution prepared by dissolving mercury in double its weight of nitric acid (specific gravity 1.42) and diluting with an equal volume of water.

In his original work Wiley used only 50 cc. of milk for some of his determinations instead of 63.75 cc., the approximate volume of the present A.O.A.C. sample, and this would be a factor influencing the amount of mercuric nitrate needed for clarification. Wiley stated in this early paper that an excess of this reagent does no harm, but he published no data showing the lactose values obtained for the same lot of milk when different quantities of this precipitating solution were used. Various workers have since shown that the use of more than 1 cc. of mercuric nitrate not only does no harm, but it is actually beneficial because higher lactose values are obtained as a result of the more complete removal of the proteins from the filtrate.

Wiley and Ewell[3] were the first to recognize the need of a larger quantity of this reagent and the desirability of a more dilute solution. They proposed diluting the mercuric nitrate solution prepared by dissolving mercury in double its weight of nitric acid with five volumes of water instead of one and using 10 cc. of this dilute solution (equivalent to 3⅓ cc. of the A.O.A.C. solution) for each 2 normal weight of milk. They also noted that the strong solution turned the precipitated protein

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 59 (1937).
[2] *Am. Chem. J.*, **6**, 289 (1884); *Analyst*, **12**, 174 (1887).
[3] *Analyst*, **21**, 182 (1896).

a slightly yellow color. Richmond and Boseley[1] also observed the coloration produced by the strong acid mercuric nitrate and expressed their preference for the dilute solution.

Richmond[2] called attention to the fact that proteins and their products of hydrolysis are usually levorotatory and will lower the estimation of milk sugar unless they are completely removed. Since phosphotungstic acid forms insoluble compounds with proteins and with proteoses, peptides, and diamino acids, which might be formed by hydrolysis of the proteins, he proposed the addition of 5 per cent phosphotungstic acid and 5 per cent of sulfuric acid (1+1) after clarification of milk with 1 cc. of acid mercuric nitrate. Higher lactose values were obtained by this method than with acid mercuric nitrate alone, the differences being greater for abnormal milks and whey powders than for normal milk and dried milks.

In 1932 Poffenberger and Almy[3] presented data that showed that 5–6 cc. of acid mercuric nitrate of the A.O.A.C. strength per 2 normal weight of milk was necessary to obtain the maximum saccharimeter readings and that an equivalent amount of the concentrated reagent diluted with three volumes of water was preferable to the strong solution. The addition of 25 cc. of 5 per cent phosphotungstic acid gave higher saccharimeter readings than were obtained by the use of acid mercuric nitrate alone, even when the larger quantities were used. They also showed the advantage of shaking the milk serum and curd for 5 minutes before filtering.

Several workers have recommended the use of larger quantities of the mercuric nitrate solution for clarifying sweetened condensed milk than the amount called for in the A.O.A.C. method for whole milk. Harrison,[4] Honegger,[5] and Richmond[6] used 3 cc. of this reagent for clarifying 26–31.4 grams of this product. Revis and Payne,[7] however, preferred to use 10 cc. of a 1 to 5 dilution of mercuric nitrate for precipitating the proteins from 26 grams of sweetened condensed milk. They state that a source of error may occur in this method of estimating sugars due to the occlusion of sugar in the precipitated curd, and that unless care is exercised the error from this source may be large. They recommend shaking the sample violently for 30 seconds after the addition of the dilute reagent in order to produce a finely divided curd and allowing the sample to stand for 5 minutes before filtering.

These investigations indicate that a larger quantity of acid mercuric nitrate is needed when determining lactose in milk than is specified by the A.O.A.C. and that a more dilute solution of this reagent is preferable to the one now recommended. The use of phosphotungstic acid in addi-

[1] *Analyst*, 22, 98 (1897).
[2] *Ibid.*, 35, 516 (1910).
[3] Paper read at the Annual Meeting of the American Dairy Science Association. Unpublished.
[4] *Analyst*, 29, 248 (1904).
[5] *Ibid.*, 51, 496 (1926).
[6] *Ibid.*, 52, 525 (1927).
[7] *Ibid.*, 39, 476 (1914).

tion to acid mercuric nitrate has also been shown to be of value in clarifying milk for a lactose determination. In order to obtain further information on the amount of mercuric nitrate needed to adequately clarify normal and abnormal milks and to ascertain what concentration of this reagent is the most desirable a series of studies on this problem was made.

TABLE 1.—*Saccharimeter readings of herd milks clarified with equivalent amounts of acid mercuric nitrate solutions of different concentration*

MERCURIC NITRATE SOLUTION		SACCHARIMETER READING*, °V.						
DILUTION	QUANTITY USED PER 2 N. WEIGHT MILK	SAMPLE 1 (PAST.)	SAMPLE 2 (PAST.)	SAMPLE 3 (RAW)	SAMPLE 4 (PAST.)	SAMPLE 5 (PAST.)	SAMPLE 6 (RAW)	SAMPLE 7 (RAW)
	cc.							
1–1	1	Turbid	9.45	Turbid	9.80	—	—	—
	2	9.70	9.75	9.40	9.93	—	—	—
	3	9.75	9.80	9.50	9.95	9.83	—	—
	4	—	—	—	10.00	9.85	—	—
	5	—	—	—	—	9.88	—	—
1–3	2	9.70	9.50	9.35	9.80	—	—	—
	4	9.75	9.73	9.40	9.98	—	—	—
	6	9.78	9.78	9.48	10.00	9.90	—	—
	8	—	—	—	9.98	9.93	—	—
	10					9.93		
1–5	3	9.75	9.65	9.40	9.78	—	Turbid	9.53
	6	9.80	9.75	9.45	9.95	—	9.15	9.58
	9	9.83	9.80	9.50	9.98	9.85	9.45	9.58
	12				10.00	9.90	9.48	9.60
	15					9.90	9.50	9.63
	18					9.90	9.55	9.68
	21						9.55	9.68
1–7	4	9.70	9.70	9.40	9.80	—		
	8	9.80	9.75	9.45	9.95	—		
	12	9.80	9.80	9.50	10.00	9.88		
	16				10.00	9.90		
	20					9.90		

* The per cent lactose in the milk is obtained by dividing the saccharimeter reading by two.

INVESTIGATIONS

Mercuric nitrate solutions of different concentration were prepared by dissolving mercury in twice its weight of nitric acid, then diluting portions of this solution with 1, 3, 5, and 7 volumes of water. Equivalent amounts of these dilutions were then used for clarifying 65.80 grams (2 normal weight) of milk. From 10 to 24 cc. of a 5 per cent solution of

phosphotungstic acid was also added to some of the milk samples to determine the effect on the apparent lactose content. The milk, precipitating reagents, and dilution water were tempered in a water bath at 23°C. before being used. The amount of milk required for a lactose determination was accurately weighed into a volumetric flask, the capacity of which had been carefully determined. The reagents were added to the milk slowly with constant agitation; the flask was filled to the mark (102.6 cc.) with water, stoppered, and shaken for 15 minutes, and the mixture was then filtered through Whatman No. 40 filter paper, the first few cc, obtained being discarded. All samples were read within one or two hours in a Bausch and Lomb saccharimeter, a 200 mm. tube being used. Two individuals made 3 to 5 readings on each sample, and the three closest values obtained by each person were averaged to secure the final value reported.

RESULTS

The apparent lactose content of several samples of herd milks clarified with equivalent amounts of acid mercuric nitrate solutions of different strengths is shown in Table 1. It will be noted that 1 cc. of the 1 to 1 dilution (A.O.A.C. recommendation) sometimes gave a turbid filtrate, and that the saccharimeter reading was always lower than that obtained when larger quantities of reagent were used for precipitation. In general, the saccharimeter reading increased to a maximum when approximately 5 cc. of the 1 to 1 dilution or equivalent quantities of the less concentrated solutions were used per 2 normal weight of milk. Two samples (6 and 7), however, required 18 cc. of the 1 to 5 dilution to yield the maximum reading.

The 1 to 1 dilution frequently gave results that were slightly lower than those obtained with equivalent amounts of the weaker solutions. These differences would probably have been more pronounced had the solution been added to the milk rapidly and with little agitation, instead of being added slowly and with considerable agitation, as in this study. In spite of these precautions the curd obtained with the 1 to 1 dilution frequently contained large lumps and was much coarser than the precipitate secured with the weaker solutions. The color of the curd varied from a grayish-brown to dark red, becoming a deeper red as the amount of reagent used increased. There was no apparent advantage of the 1 to 7 dilution over the 1 to 3 or 1 to 5 dilutions.

After standing several hours at room temperature the filtrates frequently developed a faint red color and a marked turbidity while a white sediment appeared on the bottom of the bottles. However, the filtrates obtained by using 2, 3, or 4 cc. of the 1 to 3, 1 to 5, and 1 to 7 dilutions, respectively, usually failed to show any of the above characteristics upon standing. The turbidity that developed sometimes appeared to be due

to flocculated protein and when such filtrates were refiltered the saccharimeter reading was usually slightly higher than the original value. When sufficient quantities of the acid mercuric nitrate were employed to produce the maximum optical rotation, the saccharimeter reading of the filtrate did not increase after it had been held several hours and refiltered. The red color that appeared was probably due to the action of the mercuric nitrate on protein material left in solution (Millon's reaction). The white sediment that formed was probably mercury salts precipitated by inorganic milk salts in the filtrate.

TABLE 2.—*Saccharimeter readings of normal and abnormal milks clarified with acid mercuric nitrate and phosphotungstic acid*

REAGENTS USED PER 2 N WT. MILK		SACCHARIMETER READING*, °V.						
1-5 DILUTION MERCURIC ACID	5% PHOSPHOTUNGSTIC ACID	HERD MILK	HERD MILK	MASTITIS MILK	COLOSTRUM	LATE LACTATION PERIOD	EVAPORATED (DIL. 1 TO 1.25)	RECONSTRUCTED MILK (10%)
cc.	*cc.*							
9		9.45					8.13	
12		9.48	9.70	7.10	8.73	8.50	8.25	6.83
15		9.50	9.78	7.08	8.93	8.55	8.35	6.85
18		9.55	—	7.10	9.08	8.53	—	6.90
21		9.55	—	—	—	—	—	—
12	20	—	—	7.18	—	8.55	—	6.95
15	10	—	9.83	—	9.05	—	—	
15	15	9.58	9.85	—	—	—	—	
15	20	—	9.88	7.20	9.13	8.63	8.38	6.98
15	24	9.58	9.88	—	—			

* The per cent lactose in the milk is obtained by dividing the saccharimeter reading by two

Table 2 shows the saccharimeter readings on the filtrates from normal and abnormal milks clarified with different amounts of acid mercuric nitrate (1 to 5 dilution) and 5 per cent phosphotungstic acid. From 15 to 18 cc. of the mercuric nitrate solution was required to produce the maximum saccharimeter reading. The addition of phosphotungstic acid gave slightly greater values than were obtained by the use of mercuric nitrate alone, the maximum readings being secured when 15 cc. of mercuric nitrate and 20 cc. of phosphotungstic acid were used. This increase was slightly greater for abnormal milks than for normal milks or reconstructed and evaporated milk. The filtrates obtained by clarifying the abnormal milk samples with acid mercuric nitrate developed a very deep red color after standing a few hours, but when phosphotungstic acid was used in the clarification the red color did not appear.

DISCUSSION

The data reported show that 1 cc. of acid mercuric nitrate of A.O.A.C. strength is not sufficient to adequately clarify milk for the saccharimetric determination of lactose. At least 5 cc. of this reagent is needed per 2 normal weight of milk. The present reagent is more concentrated than is desirable, because when added to milk it tends to form large lumps of curd that may occlude some of the lactose and result in a lower saccharimeter reading. A solution prepared by adding five volumes of water instead of one to the mercuric nitrate is sufficiently dilute to eliminate this source of error, provided the reagent is added to the milk slowly and with constant agitation. Approximately 15–18 cc. of this solution is needed for clarifying each 2 normal weight of milk.

Even when sufficient acid mercuric nitrate is added to secure the maximum saccharimeter reading, the reading is increased approximately 0.05–0.15 by the further addition of 20 cc. of 5 per cent phosphotungstic acid during the clarification of the milk. This difference is usually larger for various kinds of abnormal milks than it is for normal milk. The phosphotungstic acid apparently removes some of the minor proteins of milk or the products of protein hydrolysis that are not removed by the acid mercuric nitrate and this probably accounts for the increased rotatory power of the filtrate.

CONCLUSIONS

The data presented indicate—

(1) That 5–6 cc. of acid mercuric nitrate solution per 2 normal weight of milk instead of 1 cc. is required to adequately clarify milk for the determination of lactose by the optical method.

(2) That 15–18 cc. of acid mercuric nitrate solution prepared by dissolving mercury in twice its weight of nitric acid and diluting with 5 volumes of water is preferable to the use of 5 or 6 cc. of the A.O.A.C. solution.

(3) That the addition of 20 cc. of a 5 per cent solution of phosphotungstic acid per 2 normal weight of milk gives slightly higher saccharimeter readings, especially for abnormal milk, than the use of acid mercuric nitrate alone.

No report on sediment in cream and butter was given by the associate referee.

No report on naval stores was given by the referee.

No report on rosin was given by the associate referee.

REPORT ON TURPENTINE

By V. E. Grotlisch (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the last few years paint manufacturers have been developing quick drying enamels made up on a natural or synthetic resin base, which are taking the place of Duco type enamels. One of the problems they have encountered is that of finding satisfactory thinners for that type of enamel. The Standard Oil Company of New Jersey, among others, has introduced a line of thinners made from petroleum naphthas by hydrogenation. These are marketed under the trade name "Solvesso." There are four grades of these thinners, which are classified according to the use for which they are designed.

With reference to turpentine, the Association has an official method for the detection and determination of mineral oil that depends on polymerization of the turpentine with sulfuric acid. These new thinners are more or less soluble in sulfuric acid, and therefore it seems to be necessary to develop a new line of attack to detect and determine adulteration of turpentine with these new hydrogenation products. A test made by the Associate Referee on the Solvesso thinner, which most nearly approaches turpentine in characteristics, with a mixture consisting of 95 per cent turpentine and 5 per cent of the hydrogenated naphtha, gave only 1 per cent of polymerization residue, thus indicating a recovery of only 20 per cent of the adulterant, whereas the ordinary petroleum thinner gives a recovery of 70–80 per cent.

Therefore, since it appears that methods for more accurately determining the presence of these new thinners must be developed, it is recommended that the Associate Referee continue the study of methods for detection and determination of adulterants in turpentine, with special reference to the new petroleum thinners.

The Referee on Paints, Paint Materials and Varnishes gave no report but submitted the following recommendations:[1]

(1) That the methods for raw and boiled linseed oil be made official (final action).

(2) That study be made of the following additional methods of testing varnishes: Abrasion resistance, hardness, skinning tests, and alkali resistance.

(3) That study on the accelerated weather test of paints be continued.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 54 (1937).

REPORT ON ACCELERATED WEATHERING TEST OF PAINTS

By L. L. CARRICK (North Dakota Agricultural College, Fargo, N. D.), *Associate Referee*

The running of accelerated weathering tests on paints for three years indicates that the cycle used in the test must be altered to suit climatic conditions. The cycle that best suits North Dakota climate appears to be 24 hours of light followed by light and mild spray alternated with vigorous spray as follows: 24 hours of light, 20 hours of light with mild spray, 3 hours of vigorous spray; 24 hours of light, 20 hours of light with mild spray, 3 hours of vigorous spray, etc. Due to the low moisture content of woods in this climate, low temperature is found not to be a pertinent factor and refrigeration is omitted from the cycle.

Series of outside exposure texts and accelerated weathering tests on the same paints have been and are being conducted, from which definite conclusions may be expected as to a comparison of the accelerated weathering tests with natural weathering conditions.

It is recommended[1] that the study on the accelerated weathering test of paints be continued and also that, if possible, collaboration with the Sub-committee on Accelerated Weathering Tests of the American Society for Testing Materials be attempted.

No report on leathers and tanning materials was given by the referee.

No report on radioactivity was given by the referee.

REPORT ON CEREALS

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

New developments in the preparation of food products are evident continually, and cereal foods are no exception. The use of soybean flour in food products is apparently increasing. It is used with other materials in such products as bread, crackers, doughnuts, macaroni, sausage, dog foods, etc., and therefore it is desirable and sometimes necessary to have means for its detection and estimation. Some of the available procedures for the qualitative detection of soybean flour require further study, and a method for its quantitative determination is urgently needed. Accordingly, it is recommended that an associate referee be appointed to study this subject.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 54 (1937).

Macaroni may be made from flour, farina, semolina, or a mixture of any of these products. The problem of determining just what a macaroni product is made from requires more study. A recent addition to the literature in this connection is the work published by L. Borasio.[1] This paper presents the following characteristics of macaroni products: (1) Degree of cooking, (2) resistance to disintegration, (3) capacity for absorption of water, and (4) increase in volume of the paste. It seems that a combination of these physical measures, together with chemical analysis may solve this problem. Other physical measurements may also be necessary. It is recommended[2] that an associate referee be appointed to study this subject.

Some studies on the differentiation of whole wheat flour and imitation or scalped whole wheat flours have been made. As the differentiation used at present could undoubtedly be improved, it is recommended that an associate referee be appointed on this subject.

The method for the determination of original ash of phosphated and self-rising flour, *Methods of Analysis, A.O.A.C.*, 1935, has not been studied collaboratively by the Association. It is recommended that an associate referee be appointed for this work.

It is recommended that the method for the determination of fat by acid hydrolysis, *Ibid.*, 224 and 229 under "Baked Cereal Products" and "Alimentary Pastes," respectively, be adopted as official (final action).

It is recommended that the method for the extraction and identification of added color in macaroni products, *Ibid.*, 229, be adopted as official (final action).

The Referee concurs in the recommendations of the associate referees.

REPORT ON ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

One of the recommendations of the Associate Referee adopted last year was that further work be done with rapid methods of determining ash in flour, macaroni products, and baked products. Therefore the Associate Referee has given consideration to the use of other aids in ashing.

Use of Magnesium Acetate.—Especial attention was given to the magnesium acetate method published by the American Association of Cereal Chemists.[3] In this method 15 grams of magnesium acetate ($Mg(C_2H_3O_2)_2 \cdot 4H_2O$) is dissolved in denatured alcohol and made up

[1] *Giorn. risicoltura*, 25, 251 (1935).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 65 (1937).
[3] Cereal Laboratory Methods, 3rd Ed., p. 31.

to 1 liter. The solution is allowed to stand overnight and filtered if not clear. To a 3 gram sample of flour is added 3 cc. of the alcoholic solution of magnesium acetate, and after being allowed to stand for 5 minutes the sample is placed in a muffle furnace at 850° C. and allowed to flame until carbonized, when the door of the furnace is closed. Incineration is complete in 30–45 minutes. The ashing dish is then removed, cooled in a desiccator, and weighed.

The temperature (850° C.) specified in this method appears to be unnecessarily high. In order to investigate this point, samples of different cereal products were ashed by this method at the following temperatures: 850°, 800°, 775°, 700°, 650°, and 550° C. Satisfactory results were obtained at these different temperatures, but more time was required when the lower temperatures were used.

Use of Thorium Nitrate Dissolved in Alcohol-Glycerol Mixture.—Thorium nitrate was dissolved in a mixture of 40 per cent alcohol, 40 per cent glycerol, and 20 per cent water, and used in ashing both hard and soft wheat flours. The temperatures selected were the same as noted above; both platinum and porcelain dishes were employed. Again satisfactory results were obtained at the different temperatures with both kinds of dishes. Similar results were obtained with the thorium nitrate dissolved in 40 per cent alcohol, but the ashing periods were slightly longer than was the case when the thorium nitrate was dissolved in the alcohol-glycerol mixture. The experiments were extended to include hard wheat patent flour, hard wheat clear flour, white rye flour, and dark rye flour. All of these ashed satisfactorily at 850° C. when 10 cc. of the thorium-alcohol-glycerol solution was used with 3 gram samples.

When the temperature was dropped to 750° C. a 3 gram sample ashed completely in 1 hour, when 10 cc. of the thorium nitrate-alcohol-glycerol solution was used, but a 5 gram sample was incompletely incinerated in that time. No fusion of the ash was noticed under these conditions. At 800° C. and by using the thorium-alcohol-glycerol solution with 3 gram samples of white, light, and medium rye flours, the ashing was complete in 1 hour, and with 5 gram samples in 1¼ hours, but under the same conditions the ash from dark rye flours fused and accurate results were not obtained. The ashing of the dark rye was repeated, and twice as much of the thorium nitrate solution was used, but the ash still contained dark specks. When three times the regular concentration was used, there were no signs of fusion with any of the dark rye flours tested, and the ash was almost white after 1 hour at 800° C.

ASHING OF WHEAT BRAN

Wheat bran is very difficult to ash by the official A.O.A.C. method of heating not above dull redness. In this case no ashing aid is used. The color of the ash remains a dark gray even after many hours at this tem-

perature. If, however, the temperature is raised to 850° C., as recommended in the A.A.C.C. magnesium acetate method, the ash fuses before all the carbon is oxidized. At 800° C. the ash also fuses. The addition of thorium nitrate-alcohol-glycerol, even up to four times the usual quantity, did not prevent fusion of the bran ash at this temperature; but at 650° C. there was no fusion of the ash, although unburned particles were present throughout the mass even after 4 hours of heating. When 2 gram samples of bran were ashed at 700° C. the ash fused unless at least three times the usual quantity of the thorium nitrate was added.

The addition of 10 cc. of the alcoholic magnesium acetate solution (previously mentioned) to 2 grams of bran prevented fusion of the ash at 700° C. The percentage of ash was 7.12; with 15 cc. of the same solution the percentage of ash was 7.13; and with four times the usual quantity of the thorium nitrate-alcohol-glycerol the percentage was 7.17. These results were obtained after 1 hour's incineration.

COMPARISON OF MAGNESIUM ACETATE AND NITRATES OF THE RARE EARTHS

Further tests gave duplicate results with the use of the magnesium acetate solution as well as with the thorium-alcohol-glycerol when ashing wheat bran, dark rye flour, and patent wheat flour at 700° C. In the case of bran the period of incineration was extended to 1½ hours. The magnesium acetate solution as an aid in ashing was compared with the rare earth nitrates of cerium and lanthanum at 700° C. Duplicate results were obtained on wheat flour, wheat bran, and dark rye flour under these conditions.

The quantity of magnesium acetate specified in the method given in the A.A.C.C. Book of Methods yields a blank of approximately 10 mg., or nearly as great as the weight of ash from 3 grams of flour. It seemed desirable to reduce the size of this blank if equally good results could be obtained. Accordingly a solution was made by dissolving 9 grams of magnesium acetate ($Mg(C_2H_3O_2)_2 \cdot 4H_2O$) in 1 liter of 95 per cent alcohol instead of 15 grams; 3 cc. of this solution yielded a blank of approximately 5 mg.

When ashing at 700° C. this more dilute acetate solution gave entirely satisfactory results. In ashing bran the size of the sample is reduced to 1 gram, and two or three times the regular quantity of the magnesium acetate solution, namely 6 or 9 cc., is required to prevent fusion of the ash. A collaborative study was made with this modified magnesium acetate method. The modifications consisted of reducing the concentration of magnesium acetate from 15 to 9 grams per liter and reducing the temperature from 850° to 700° C.

Wheat flour, white rye flour, and semolina were ashed by seven collaborators (Table 1).

TABLE 1.—*Collaborative ash results*

COLLABORATOR	ASH FROM—		
	WHEAT FLOUR	WHITE RYE FLOUR	SEMOLINA
	per cent	*per cent*	*per cent*
Jos. A. Mathews	0.399	0.690	0.662
V. E. Munsey	0.449	0.750	0.697
L. H. Bailey	0.437	0.730	0.682
J. T. Kiester	0.453	0.709	0.676
W. O. Winkler	0.420	0.725	0.677
J. Davidson	0.445	0.750	0.687
H. C. Fellows	0.408	0.700	0.664
Average	0.430	0.722	0.678

The variation in wheat flour ash results was 0.054 per cent; in the white rye flour ash, 0.060 per cent; and in the semolina ash, 0.035 per cent. These variations might be lessened with more experience in the use of the method on the part of the collaborators. Fellows ashed the same collaborative samples by the official A.O.A.C. method and obtained 0.406 per cent for the wheat flour, 0.710 per cent for the white rye, and 0.663 per cent for the semolina. These are very close duplicate values to those he obtained by the modified magnesium acetate method.

One of the most probable sources of error with this method is in not adding exactly the same quantity of the acetate solution to each sample as is used in the blank. For this reason the use of a buret is suggested for measuring the alcoholic acetate solution. Other changes that have been made in the method are the use of approximately 90 per cent alcohol in place of the 95 per cent alcohol specified in the original method, and an adjustment of the concentration of the magnesium acetate so that 5 cc. of the solution will yield approximately 5 mg. of magnesium oxide.

The reason for changing to 90 per cent alcohol is that the magnesium acetate is not readily soluble in the 95 per cent alcohol, but it may first be dissolved in a little water and then the alcohol added to form a clear solution.

If 5 cc. of the solution is used, all the flour (3 grams) will be saturated without stirring the sample. The excess alcohol may be evaporated or burned off without spattering. When ashing bran, wheat germ, etc., 10 cc. of the magnesium acetate solution should be taken for every 1 gram sample.

Wheat germ, noodles, bread, and bran were ashed at 700° C. by using 10 cc. of the thorium nitrate and magnesium acetate solutions, respectively. The blanks on these solutions were 10 mg. of thorium oxide and 10.4 mg. of magnesium oxide. The results are shown in Table 2.

TABLE 2.—*Ash values with thorium nitrate and magnesium acetate*

ASHING AID	ASH FROM—			
	1 g. WHEAT GERM	3 g. NOODLES	3 g. BREAD	1 g. BRAN
Thorium nitrate	3.68*	0.73	1.90	6.69
Magnesium acetate	3.47	0.75	1.95	6.57

* Fused—dark specks.

There was no indication of fusion of the ash from the wheat germ when magnesium acetate was used as the aid. Ash from the other materials appeared to be equally satisfactory.

The conclusions to be drawn from this work are that an alcoholic solution of thorium nitrate or other alkaline earth nitrate is satisfactory as an ashing aid for cereal products and that magnesium acetate is likewise satisfactory for the same purpose. The latter has the advantage of being a more common chemical and seems to be a little more efficient in preventing fusion of the ash. The method as developed was published in *This Journal*, **20**, 69 (1937).

It is recommended[1] that the modified magnesium acetate method outlined be adopted as a tentative method, and that further work be done with this method as a rapid method for ashing cereal products.

REPORT ON H-ION CONCENTRATION

By GEORGE GARNATZ (The Kroger Food Foundation, Cincinnati, Ohio), *Associate Referee*

All the recommendations made last year by the Associate Referee covering the determination of H-ion concentration of flour, baked products, and alimentary pastes were followed with one exception, that regarding conditions of light for properly making color comparisons.

While light studies were undertaken, limited time and unforeseen obstacles render any report on this phase of the work, in its present state, premature.

After careful thought the method was rewritten to cover all the details listed in the 1935 report except the light conditions under which color comparisons should be made, and is as follows:—

PREPARATION OF INDICATOR SOLUTIONS[2]

(a) *Gamma (2:5) dinitrophenol.*—Dissolve 0.05 gram of indicator in 100 cc. of recently boiled distilled water.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 66, 69 (1937).
[2] Modification of directions given in "Determination of Hydrogen Ions" by W. M. Clark, 3rd Ed, pp. 192–202.

(b) *Paranitrophenol.*—Dissolve 0.10 gram of indicator in 100 cc. of recently boiled distilled water.

(c) *Metanitrophenol.*—Dissolve 0.30 gram of indicator in 100 cc. of recently boiled distilled water.

PREPARATION OF STOCK SOLUTIONS

0.2*M* acid potassium phosphate (KH_2PO_4)
0.2*M* acid potassium phthalate ($KHC_8H_4O_4$)
0.2*M* boric acid with 0.2*M* potassium chloride (H_3BO_3, KCl)
0.2*M* sodium hydroxide (NaOH) and 0.2*M* hydrochloric acid (HCl)

The water used should be recently boiled distilled water.

(a) *Acid potassium phthalate solution.*—Dry the C. P. salt to constant weight at 110°–115° C. A 0.2*M* solution contains 40.836 grams of the salt in 1 liter of solution.

(b) *Acid potassium phosphate solution.*—Dry the C. P. salt to constant weight at 110°–115° C. A 0.2*M* solution should contain 27.232 grams of the salt in 1 liter of solution. The solutions should be distinctly red with methyl red, and distinctly blue with bromphenol blue.

(c) *Boric acid, KCl solution.*—Dry the C. P. boric acid to constant weight in a desiccator over $CaCl_2$. Dry the C. P. potassium chloride in an oven at 115°–120° C. for 2 days. One liter of the solution should contain 12.405 grams of boric acid and 14.912 grams of KCl.

(d) *Sodium hydroxide solution.*—This solution is the most difficult to prepare since it should be as free as possible from carbonate. Dissolve 100 grams of NaOH in 100 cc. of distilled water and allow to stand overnight till the carbonate has settled. Pipet the clear solution from the sediment and dilute to a solution somewhat more concentrated than normal. Standardize this solution with an acid solution of known strength, or with a sample of acid potassium phthalate. From this approximate standardization calculate the amount required to furnish a 0.2*M* solution. Make the required dilution with the least possible exposure and pour the solution into a Pyrex glass bottle. The solution should now be most carefully standardized. The simplest method of doing this is by means of the acid potassium phthalate. 0.04084 grams of acid potassium phthalate is equivalent to 1 cc. of 0.2*M* NaOH. It is preferable to use a factor with the solution rather than attempt adjustment to an exact 0.2*M* solution. Use phenolphthalein as the indicator.

PREPARATION OF BUFFER SOLUTIONS

The maximum range of standard buffer solutions usually needed in cereal work is from *p*H 5.0 to *p*H 8.6. Prepare these from the following stock solutions, and in each case dilute to 200 cc.

Phthalate-NaOH Mixtures

*p*H	0.2 *M*	0.2 *M*
5.0	50 cc. KHPhthalate	23.65 cc. NaOH
5.2	50 cc. KHPhthalate	29.75 cc. NaOH
5.4	50 cc. PHPhthalate	35.25 cc. NaOH
5.6	50 cc. KHPhthalate	39.70 cc. NaOH
5.8	50 cc. KHPhthalate	43.10 cc. NaOH
6.0	50 cc. KHPhthalate	45.40 cc. NaOH
6.2	50 cc. KHPhthalate	47.00 cc. NaOH

KH_2PO_4-NaOH Mixtures

*p*H	0.2 *M*	0.2 *M*
5.8	50 cc. KH_2PO_4	3.66 cc. NaOH
6.0	50 cc. KH_2PO_4	5.64 cc. NaOH
6.2	50 cc. KH_2PO_4	8.55 cc. NaOH
6.4	50 cc. KH_2PO_4	12.60 cc. NaOH
6.6	50 cc. KH_2PO_4	17.74 cc. NaOH
6.8	50 cc. KH_2PO_4	23.60 cc. NaOH
7.0	50 cc. KH_2PO_4	29.54 cc. NaOH
7.2	50 cc. KH_2PO_4	34.90 cc. NaOH
7.4	50 cc. KH_2PO_4	39.34 cc. NaOH
7.6	50 cc. KH_2PO_4	42.74 cc. NaOH
7.8	50 cc. KH_2PO_4	45.17 cc. NaOH
8.0	50 cc. KH_2PO_4	46.85 cc. NaOH

Boric Acid, KCl-NaOH Mixtures

*p*H	0.2 *M* 0.2 *M*	0.2 *M*
7.8	50 cc. H_3BO_3, KCl	2.65 cc. NaOH
8.0	50 cc. H_3BO_3, KCl	4.00 cc. NaOH
8.2	50 cc. H_3BO_3, KCl	5.90 cc. NaOH
8.4	50 cc. H_3BO_3, KCl	8.55 cc. NaOH
8.6	50 cc. H_3BO_3, KCl	12.00 cc. NaOH

PREPARATION OF COLORIMETRIC STANDARDS

Place 20 cc. of the buffered solution in ampuls ¾″ in diameter, or in test tubes of similar bore, and add 0.5 cc. of indicator solution. The useful *p*H ranges of the indicators are:

Gamma 2:5 dinitrophenol	4.0–5.5
Paranitrophenol	5.2–7.0
Metanitrophenol	6.7–8.4

METHOD OF MEASURING *p*H

To 10 grams of the sample add 100 cc. of cool, recently boiled distilled water and digest at 25° C. for ½ hour, shaking occasionally during the digestion period. Allow the mixture to stand quietly for 15 minutes[1] and then decant the supernatant liquid through a folded hardened dry filter paper. Discard the first 5 cc. to come through, but catch the next 20 cc. in a tube exactly like the tubes holding the colorimetric standards. Add 0.5 cc. of the proper indicator and compare the resultant color with the prepared standards to get the *p*H.

A somewhat crude but helpful application of Walpole's principle to compensate for color and turbidity of the sample can be made from a block of wood. Six deep holes, each large enough to hold one color standard or sample tube, are bored parallel to one another in pairs. Adjacent pairs are placed as close to one another as possible without breaking through the intervening walls. Perpendicular to these holes and running through each pair are bored *smaller* holes, through which the test tubes may be viewed.

The center pair of test tubes holds the solution to be tested plus the indicator and also a water blank. At either side are placed the standards

[1] Increased from 10 to 15 minutes in recognition of comments made on presentation of 1935 report.

colored with the indicator, and each is backed by a sample of the solution under test. The light is placed on the side of the comparator containing the two controls and water blank. If there be placed over the comparator holes a ground glass and a glass of cobalt blue, the color quality of two tubes will be very different when there is no match. This increases the differentiating ability of the eye, and makes the use of nitrophenols with colored solutions much more satisfactory.

EXPERIMENTAL

As in the previous year's work, samples of flour, bread, cake, macaroni, and crackers were sent out to a number of collaborators, together with the directions given previously. Adequate quantities of the three indicators were also furnished. It was requested that the various solutions, color standards, and *p*H measurements be made by following the directions exactly, and that all *p*H measurements be made at least in duplicate.

The following individuals participated in these tests:

(1) H. Adler, Victor Chemical Works
(2) G. L. Alexander, Commercial Milling Co.
(3) H. V. Moss, Monsanto Chemical Co.
(4) H. M. Simmons, Midwest Laboratories Co., Inc.
(5) W. Tholstrup, Pillsbury Flour Mills Co.
(6) L. M. Thomas, Keever Starch Co.
(7) W. Reiman, Kroger Food Foundation

Several other individuals were invited to collaborate, but due to various reasons they were unable to submit reports.

The results reported by the several collaborators were as follows:

COLLABORATOR	CRACKERS	CAKE	BREAD	MACARONI	FLOUR
1	7.5 7.5	7.6 7.6	5.3 5.2	5.8 5.8	5.5 5.6
2	7.3 7.3	7.6 7.6	5.1 5.1	5.6 5.6	5.5 5.5
3	7.7 7.7	7.6 7.65	5.1 5.1	5.9 5.8	5.6 5.55
4	7.3	7.4	5.1	5.7	5.4
5	7.8*	8.0*	5.4*	6.2*	5.8*
6	7.3 7.4	7.4	5.0 4.9	5.6	5.4
7	7.3 7.4	7.6 7.6	5.2 5.3	6.0 5.9	5.5 5.4
Average	7.41	7.54	5.13	5.75	5.48
Range	0.4	0.25	0.40	0.40	0.20
Electrometric[1]	7.59	7.54	5.22	5.97	5.68
Electrometric[2]	8.11	7.59	5.17	5.83	5.53

* Omitted from Average.
[1] Determined by W. Reiman.
[2] Determined by H. V. Moss.

DISCUSSION

In contrast to last year's work, the collaborators were requested to prepare all solutions in connection with the test, and in spite of this the range of variation in results is certainly no greater and may even be considered less, which is an encouraging indication. Moreover, in comparison with

the results obtained electrometrically the colorimetric values show quite satisfactory agreement.

The two electrometric measurements of *p*H for the crackers were so seriously at variance with each other that an attempt was made to ascertain the cause. As a result another possible source of error in the application of the test was indicated by the observation that *p*H progressively decreased with the passage of time between filtration and *p*H measurement. In all probability this is due to bacterial action and is illustrated by the data tabulated below for crackers.

TIME INTERVAL	AFTER FILTRATION	30 MIN. LATER	1 HR. LATER	3 HRS. LATER	24 HRS. LATER
*p*H	7.90	7.64	7.68	7.58	5.19

While this decrease in *p*H is characteristic of all the cereal products tested, it is most noticeable in the instance of crackers, where the *p*H seemingly is more favorable to bacterial development and hence time is a more critical factor.

Some attempts were made in an effort to stabilize the filtered extracts against this development, but all such attempts were unsuccessful and time did not permit further investigation.

In substance the comments of the several collaborators were not adverse criticisms of the method, its manipulation, or the results obtained, but they were directed at the difficulties encountered through the use of the three nitrophenol indicators. That the color changes between *p*H intervals of 0.2 were difficult to perceive and that color intensities were somewhat faint were two outstanding criticisms.

This was followed up by questioning the collaborators as to the indicators they customarily used to cover the range for cereal products and it was found that the following were in rather general use:—Bromcresol green, 3.8–5.4; methyl red, 4.2–6.3; chlorphenol red, 4.8–6.4; bromthymol blue, 6.0–7.6; phenol red, 6.8–8.4; cresol red, 7.2–8.8. Additional comments indicated that unfamiliarity with the nitrophenol indicators was no small factor in contributing to criticism of their use. A suggestion was forthcoming to the effect that some relief might be obtained through the use of a greater quantity of indicator solution even to the extent of as much as four times the volume specified in the directions.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method be subjected to further study.

(2) That the "10 minutes" in the directions reading, "Let the flask stand quietly for 10 minutes," be changed to read "15 minutes."

(3) That studies relative to light conditions for properly making color comparisons be continued.

(4) That studies on stabilizing filtered extract be investigated further.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 65 (1937).

(5) That the desirability of using a greater amount of the nitrophenol indicators be investigated.

(6) That collaborative studies comparing bi-color indicators with the nitrophenol indicators be continued.

ACKNOWLEDGMENT

The Associate Referee desires to express his appreciation for the interest and cooperation extended by the several collaborators. Acknowledgment is also made of the work done by W. Reiman in conducting the various tests and in preparing this report.

REPORT ON STARCH IN FLOUR

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In accordance with last year's recommendation the modified Rask, *Methods of Analysis, A.O.A.C.*, 1935, 213, and the modified Mannich-Lenz method, *J. Nat. Research Council of Canada*, **11**, 751 (1934), were submitted to collaborators. The three samples studied were corn meal, wheat flour, and rye flour, marked No. 1, No. 2, and No. 3, respectively. Previous collaborative study with the Rask method had been limited to wheat flour, whole wheat flour, and gluten flour.

Table 1.—*Collaborative results*

ANALYST	MODIFIED RASK METHOD	MODIFIED MANNICH-LENZ METHOD
	Sample No. 1	
	per cent	*per cent*
1	63.90	69.03
	63.88	69.03
		69.31
	Av. 63.89	69.23
		Av. 69.15
2	64.04	66.91
	64.72	66.61
	63.44	67.87
	64.90	67.15
	63.68	
		Av. 67.13
	Av. 64.16	
3	64.90	67.38
	64.52	67.28
	Av. 64.71	Av. 67.33

TABLE 1.—*Collaborative results* (*Continued*)

ANALYST	MODIFIED RASK METHOD	MODIFIED MANNICH-LENZ METHOD
	per cent	*per cent*
4	54.4	66.5
	51.1	
	Av. 52.8	
5	61.3	gel-like and unfilterable after digestion
6	61.8	gel-like and unfilterable after digestion
	Max. 64.71	Max. 69.15
	Min. 61.3	Min. 66.5
	Av. 63.17	Av. 67.53
	Sample No. 2	
1	73.90	76.42
	74.20	75.81
	73.60	Av. 76.11
	Av. 73.90	
2	76.00	72.26
	74.10	73.58
	72.50	72.93
	73.52	73.85
	73.40	73.56
	74.12	Av. 73.24
	Av. 73.94	
3	74.47	73.65
	74.61	73.89
	Av. 74.54	Av. 73.77
4	54.5	72.8
	62.2	
	Av. 58.35*	
5	73.5	75.2
		74.3
		Av. 74.8
6	75.1	73.7
	Max. 75.1	Max. 76.11
	Min. 73.5	Min. 72.80
	Av. 74.20	Av. 74.07

* Past experience with collaborative results by the modified Rask method indicates the results of Analyst 4 should not be considered.

TABLE 1.—*Collaborative results (Continued)*

ANALYST	MODIFIED RASK METHOD	MODIFIED MANNICH-LENZ METHOD
	Sample No. 3	
	per cent	*per cent*
1	70.06	71.40
	69.64	69.94
	Av. 69.85	Av. 70.67
2	71.28	68.37
	69.39	68.13
	69.83	65.44
	Av. 70.17	Av. 67.31
3	69.56	68.19
	69.52	67.92
	Av. 69.54	Av. 68.05
4	Acid dispersion could not be filtered	67.6
5	70.3	67.0
		65.0
		65.5
		Av. 65.8
6	69.7	60.0
	Max. 70.3	Max. 70.67
	Min. 69.54	Min. 60.0
	Av. 69.91	Av. 66.59

Analyst 5 changed the modified Mannich-Lenz method by digesting in an autoclave at 15 pounds pressure, which corresponds to a temperature of 250° F., because of excessive frothing during the digestion in the submitted method and also because digestion was incomplete, the residue giving a positive test for starch. Instead of adding 2 cc. of 0.8 per cent acetic acid as specified in the submitted method he added 6 cc., which was necessary to obtain a clear dispersion. Mechanical shaking with 100 cc. of alcohol was also found to be a distinct advantage. The results are shown in Table 2.

TABLE 2.—*Results reported by Analyst 5*

	per cent		*per cent*		*per cent*
Sample No. 1	65.7	Sample No. 2	72.4	Sample No. 3	65.5
	65.5		70.8		65.0
	Av. 65.6		Av. 71.6		Av. 65.3

DISCUSSION OF RESULTS AND COMMENTS

Variation in the average values on corn meal, Sample No. 1, by the modified Rask method is too great, but the agreement among collaborators on wheat flour, Sample No. 2, and rye flour, Sample No. 3, is very good. The agreement by the modified Mannich-Lenz method is not so good as by the modified Rask method.

The average value for collaborators using the Mannich-Lenz method was 4.3 per cent higher than that by the modified Rask method on corn meal (sample No. 1), while the reverse was true on rye flour (sample No. 3), the difference being 3.3. per cent. The average values by the two methods on wheat flour were the same.

The revision of the modified Mannich-Lenz method used by Analyst 5 should be further studied. Apparently some revision of this method is necessary. Nearly all collaborators reported difficulty with frothing during digestion and also difficulty in filtering. Substitution of a beaker for a flask avoids much of the frothing difficulty. As an aid in filtration Collaborator Carson filters through a dry Jena sintered crucible, size G3, half filled with dry fluffy asbestos. He states that stirring alone in the modified Rask method was not sufficient to remove all hydrochloric acid during the alcohol washings, but that after precipitation with alcohol, if the starch precipitate is thoroughly broken up by pressing against the side of the beaker and tamping with a stirring rod no difficulty is encountered through incomplete removal of the hydrochloric acid. If hydrochloric acid is not completely removed from the starch precipitate the starch result is wrong.

The results on cereal flours by the modified Rask method given in this report and in previous reports on collaborative studies by this method indicate that fairly satisfactory results can be obtained. The great variation occasionally reported is due to inexperience of the analyst.

Some experimental work was done by the Referee on the application of the J. T. Sullivan method, *This Journal*, **18**, 621 (1935), to food products, but the slight revisions made in the method are not sufficiently conclusive to report. The method is specific and easily manipulated, and would have the advantage of wide application to plants, feeds, and foods. However the method is quite long, and only 8 or 10 samples can be completed in 2½ days.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative study be made of the application of the modified Rask method to products not already studied.

(2) That further collaborative study be made of the Mannich-Lenz method, provided the frothing and filtering difficulties can be eliminated.

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 65 (1937).

(3) That further study be made of the application of the Sullivan method to food products such as bread, cake, crackers, etc., as well as cereals.

REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

The study of the method for the detection of benzoyl peroxide in flour was continued this year, but when submitted to three collaborators the modifications were not found to be satisfactory.

The method as modified last year was then submitted without change, as the results of the collaborators had indicated that it might be quantitative. No more time could be devoted to study this year.

The results obtained from four collaborators, which confirmed those of last year, show that the method as modified is a satisfactory qualitative method. The quantitative results are disappointing, but the Associate Referee is convinced that with further study the procedure can be made a reliable quantitative method.

All collaborators are members of the U. S. Food and Drug Administration.

Mg. of benzoyl peroxide per kilo of flour

COLLABORATOR	SAMPLE A		SAMPLE B		SAMPLE C	
	PRESENT	RECOVERED	PRESENT	RECOVERED	PRESENT	RECOVERED
J. S. Ard, New York	15	7.3	0	0	30	13.3
M. S. Gnagy, Los Angeles	15	0.8	0	0	30	4.4
	15	2.4	0	0	30	4.0
J. L. Hogan, New York	15	8.0	0	0	30	17.0
Manuel Tubis, Philadelphia	15	5.6	0	0	30	9.2

The small recoveries obtained by Gnagy may be accounted for by the fact that one month elapsed before he was able to complete the method. The ether extract of the distillate, made alkaline and allowed to stand in acetone for a week, developed a tar-like material that probably influenced the recovery of benzoic acid.

The second recommendation of the committee, that the methods for the determination of chlorine bleach published in *This Journal*, **18**, 489 (1935) be studied collaboratively, could not be carried out because of lack of time.

It is recommended[1] that the method for the detection of benzoyl peroxide bleach in flour be further studied for the purpose of making it quantitative.

REPORT ON CARBON DIOXIDE IN SELF-RISING FLOUR

By HOWARD ADLER (Victor Chemical Works, Chicago, Ill.), *Associate Referee*

The last collaborative work on the determination of carbon dioxide in self-rising flour was undertaken in 1931, *This Journal*, **15**, 588 (1932), and the method was studied further by L. D. Whiting, the last referee, in 1932, *Ibid.*, 16, 511 (1933). At that time a gasometric method was employed, and it was found that the recovery of carbon dioxide was not complete. The variation in recovery of carbon dioxide was from 93.8 to 95.5 per cent of that present in the flour, and the recommendation was made that in future work some attempt be made to determine the factor required to convert the carbon dioxide recovered to carbon dioxide present. This year some preliminary collaborative work was undertaken with this in mind.

One sample of self-rising flour was carefully prepared from the usual ingredients (salt, monocalcium phosphate, sodium bicarbonate, and an average grade of soft wheat flour that had a moisture content of 11.3 per cent, protein 9.38 per cent, and ash 0.373 per cent. The carbon dioxide content calculated from the proportion of sodium bicarbonate used in this self-rising flour was 0.7455 per cent. The Associate Referee restricted his work to one sample this year because the previous collaborative work had not shown any great variation in the efficiency of the method as applied to different grades of self-rising flour. This sample was sent to seven laboratories and also studied in the laboratory of the Associate Referee. The collaborators were asked to report their results on the sample after using the following methods:

Method A.—The method used in the 1931 A.O.A.C. collaborative work, *This Journal*, **15**, 588 (1932).

Modified Method A.—Same as Method A except that instead of the factor weight of self-rising flour being determined from the barometric pressure and temperature at the time the sample was weighed being used, 17.00 grams of the flour was used and the result was corrected to the factor weight for the barometric pressure and temperature at the time the gas volume was read.

Method B.—Apparatus and reagents same as those used in Method A, and method of calculating results same as that in Modified Method A. The chief advantage of this method is the shorter time required. It is a method that has been in use in many laboratories for the control of self-rising flour when time is an important factor. The determination by this method is as follows:

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 66 (1937).

Weigh 17 grams of the sample into the decomposition flask. Place the sample and approximately 20 dry glass beads in the dry decomposition flask and connect with the apparatus. Open the T-tube stopcock, and by means of the leveling bulb bring the displacement solution to the zero mark. Allow the apparatus to stand 1–2 minutes to insure equalization of temperature and pressure within the apparatus with those of the room. Close the stopcock, lower the leveling bulb, and run 50 cc. of dilute sulfuric acid through the 25 cc. graduated tube into the decomposition flask. Keep the displacement solution in the leveling bulb at all times during the decomposition at a lower level than that in the gas measuring tube to prevent the liberated CO_2 from escaping through the acid buret into the air. Rotate, and then vigorously agitate the decomposition flask for about 3 minutes to secure intimate mixture of the contents. Allow to stand for 3 minutes. Equalize the pressure in the measuring tube by means of the leveling bulb, and read the volume of gas evolved, allowing 50 cc. for acid used.

The collaborative results obtained by these methods are as follows:

Method A

COLLABORATOR	DATE (1936)	TEMP.	BAR. PRESS.	FACTOR WT.	CO_2 EVOLVED	AVERAGE CO_2
		°C.	mm.	gram	per cent	per cent
L. H. P.	9/4	28	750		0.720	
Monsanto Chemical					0.710	0.715
Co., St. Louis						
E. McK.	9/4	29.5	748		0.715	
Monsanto Chemical					0.710	0.713
Co., St. Louis						
L. H. Bailey		28	768	17.338	0.70	
U. S. Bur. of Chem. and					0.71	
Soils, Washington					0.70	0.703
George Garnatz	10/1	25	752	17.254	0.70	
Kroger Food Founda-					0.72	0.71
tion, Cincinnati						
L. D. Whiting		25.5	759.4	17.380	0.798	
Ballard and Ballard		24.5	756	17.395	0.780	
Louisville, Ky.		24.5	756	17.395	0.780	0.786
H. W. Putnam	9/14	34.4	757	16.42	0.757	
Igleheart Bros. Inc.		34.4	757	16.42	0.762	0.759
Evansville, Ind.	10/28	25.6	754	17.25	0.710	0.710
W. T. Tholstrup	9 /29	26	742.4	16.93	0.67	0.67
Pillsbury Flour Mills						
Minneapolis						
B. Jordan	10/2	22.3	733.3	17.052	0.70	
State Chem. Lab.	10/2	22.3	733.3	17.052	0.71	
Vermillion, S. D.	10/3	21.0	744.0	17.433	0.70	
	10/3	21.0	744.0	17.433	0.70	
	10/3	23.0	744.0	17.250	0.695	
	10/3	23.0	744.0	17.250	0.695	0.700
Average						0.718

Modified Method A

COLLABORATOR	DATE (1936)	TEMP.	BAR. PRESS.	CO_2 EVOLVED	AVERAGE CO_2
		°C.	mm.	per cent	per cent
George Garnatz	10/1	24.5	751.7	0.70	
		25.0	752	0.70	0.70
L. D. Whiting		26.0	759.4	0.805	
		25.0	755.9	0.788	
		24.75	755.9	0.789	0.794
H. W. Putnam	9/15	33.9	757	0.755	
	9/15	35.6	757	0.748	
	9/15	37.2	757	0.740	0.748
	10/28	27.2	754	0.694	
	10/28	26.7	754	0.706	0.700
B. Jordan	10/3	23	744	0.692	
	10/3	23	744	0.698	
	10/3	23	744	0.695	
	10/3	23	744	0.695	0.695
J. Schlaeger	8/28	28	744	0.710	
Victor Chemical	8/28	29	744	0.696	
Works, Chicago	8/31	29	750	0.727	
	8/31	30	750	0.732	
	9/3	29	749	0.701	
	9/3	29.5	749	0.713	0.713
Average					0.725

Method B

COLLABORATOR	DATE (1936)	TEMP.	BAR. PRESS.	CO_2 EVOLVED	AVERAGE CO_2
		°C.	mm.	per cent	per cent
L. H. P.	9/4	28	750	0.721	
	9/4	28	750	0.710	0.715
E. McK.	9/4	29.5	748	0.705	
	9/4	29.5	748	0.703	0.704
L. H. Bailey		28	768	0.714	
		28	768	0.714	
		28	768	0.704	0.710
George Garnatz	10/1	25	752	0.70	
		25	752	0.69	0.695
L. D. Whiting		27.25	758.8	0.789	
		24.75	755.9	0.784	
		24.75	755.9	0.782	0.785

Method B (Continued)

COLLABORATOR	DATE (1936)	TEMP.	BAR. PRESS.	CO_2 EVOLVED	AVERAGE CO_2
		°C.	mm.	per cent	per cent
H. W. Putnam	9/17	29.4	757	0.698	
	9/17	29.4	757	0.698	
	9/17	29.4	757	0.698	
	10/28	27.8	754	0.691	
	10/28	27.8	754	0.696	0.696
W. T. Tholstrup	9/29	26	742.4	0.668	0.668
B. Jordan	10/3	21	746	0.694	
	10/3	21	746	0.689	
	10/3	21	746	0.689	
	10/3	21	746	0.689	0.690
J. Schlaeger	8/28	28.5	744	0.703	
	8/28	29	744	0.696	
	8/31	30.5	750	0.715	
	8/31	31	750	0.718	
	9/3	29.5	750	0.714	
	9/3	29.5	750	0.709	0.710
R. A. Barackman	9/9	20.2	749	0.685	
Victor Chemical		20.3		0.685	
Works, Chicago		25.3		0.687	
		25.3		0.682	
		29.8		0.692	
		30.2		0.697	
		34.8		0.705	
		35.0		0.705	
		40.0		0.715	
		40.5		0.710	0.697
Average					0.707

DISCUSSION OF RESULTS

In the collaborative work of 1931 the average amount of CO_2 recovered was from 93.8 to 95.5 per cent theoretical. In the work here reported the averages are: Method A, 96.3 per cent; Modified Method A, 97.2 per cent; and Method B, 94.7 per cent. Many of the individual determinations are as close together as one could reasonably expect from a method of this type. There is, however, too great a deviation from the mean to make it possible at this time to determine the correct factor for the conversion of carbon dioxide recovered to carbon dioxide present.

In addition to running the samples according to the instructions issued, some work was carried out in a modified gasometric apparatus where

the temperature could be controlled. In this apparatus determinations were made covering the range of temperature from 20° to 40° C. While the results show a slight tendency to be higher at the higher temperatures, the increase in carbon dioxide recovered was not great. The average recovery is not far from the average of the other collaborators.

The gasometric method appears to give results that are satisfactory for most practical purposes. It is recommended,[1] however, that this method for the determination of carbon dioxide be given further study, and that Method A be used, as proposed by the previous referee on this subject, modified to use a 17 gram sample. If this work is continued, an attempt will be made to obtain results from a larger number of collaborators and to determine what variables influence the carbon dioxide recovery.

REPORT ON UNSAPONIFIABLE CONSTITUENTS, BAKED AND MACARONI PRODUCTS

By E. O. Haenni (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In 1935 Committee C recommended that the studies on unsaponifiable matter in flour, bread, and macroni be discontinued and instead methods for the determination of sterols in conjunction with similar work on eggs be studied. The determination of unsaponifiable constituents in these cereals is at present only significant in the evaluation of the correction to be used in estimating the egg solids content of noodles and similar egg products. Accordingly, the methods have been developed with this use of the results in mind and the report of the work is to be included in a future report on the unsaponifiable constituents of eggs and egg products.

It is therefore recommended[2]—

(1) That further direct studies on the determination of unsaponifiable constituents of flour, and baked and macaroni products be discontinued.

(2) That development of methods for the determination of the sterol content of cereals, if such data become necessary to the evaluation of the egg content of farinaceous products, be carried on in conjunction with the same work on eggs.

REPORT ON MILK SOLIDS IN MILK BREAD

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year it was recommended that collaborative work be continued. Accordingly, samples were again submitted to collaborators to be

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 66 (1937)
[2] *Ibid.*

analyzed by the same methods as were submitted last year, namely, the citric acid method and the so-called fat method. These samples of bread (baked by L. H. Bailey, Bureau of Chemistry and Soils) were sent out marked No. 1, No. 2, and No. 3, and they contained, respectively, no milk solids, 3.9 per cent, and 7.7 per cent of milk solids on the moisture-free basis.

Collaborative results on milk solids

ANALYST	H_2O	CITRIC ACID METHOD, M.F.B.	FAT M.F.B.	FAT NO.	FAT METHOD, M.F.B.
			Sample No. 1		
	per cent	*per cent*	*per cent*		*per cent*
I	10.6	0.59	4.33	1.10	0.0
		0.60	4.38	1.06	
			Av. 4.36		
II	9.8	0.11	3.06	2.47	0.48
III	10.8	0.11	4.30	4.16	1.45
IV	10.5	0.00	4.31	4.18	
			4.28	3.62	
			Av. 4.30	3.90	1.33
V	10.7	0.32	4.19	1.52	
		0.11	4.28	1.60	
		0.22	Av. 4.24	1.56	0.25
VI	10.5	0.00	3.38	4.03	1.09
			Sample No. 2		
I	9.7	4.30	5.53	8.49	4.36
		4.56	5.55	8.38	
		4.43	5.54	8.44	
II	8.9	4.07	4.41	11.39	4.87
III	10.1	3.53	5.56	9.38	4.95
IV	9.7	3.58	5.50	12.21	
			5.54	12.72	6.72
			5.52	12.47	

Collaborative results on milk solids (Continued)

ANALYST	H_2O	CITRIC ACID METHOD, M.F.B.	FAT M.F.B.	FAT. NO.	FAT METHOD, M.F.B.
	per cent	*per cent*	*per cent*		*per cent*
V	9.9	3.59	5.42	8.49	
		3.65	5.33	8.65	4.33
		——	——	——	
		3.62	5.38	8.57	
VI	9.9	0.02	3.77	10.24	3.7
		Sample No. 3			
I	10.0	8.10	6.44	12.80	
		7.92	6.42	12.84	8.08
		——	——	——	
		8.01	6.43	12.82	
II	9.6	6.93	4.83	11.58	5.44
III	10.4	6.98	6.44	13.17	8.30
IV	10.1	7.20	6.48	15.43	
		7.64	6.46	15.29	9.88
		——	——	——	
		7.42	6.47	15.36	
V	10.3	7.01	6.35	9.66	
		6.35	6.29	10.35	6.04
		——	——	——	
		6.68	6.32	10.00	
VI	10.3	1.41	5.16	14.12	7.2

DISCUSSION OF RESULTS AND COMMENTS

On the basis of last year's and this year's collaborative results it seems legitimate to disregard all the results of Analyst VI and the results by Analyst II on the fat method. The remaining results for the determination of milk solids by the citric acid method are reasonably close to the true value. The results for milk solids calculated from fat content are unsatisfactory, due principally to the great variation in the results on the so-called fat number. It may be noted that Analysts I, III, IV, and V obtained almost identical results for total fat. This same condition occurred last year. The variation in fat number by Analysts I, III, IV, and V is because of faulty technic in running the fat number on the fat or the extracted fat had a different composition. Before any more work is done on this method, several oils or fats of different fat number should be analyzed by these collaborators to determine the variation that occurs when

fats of the same composition are analyzed. If uniform results are obtained, it will then be necessary to add a known amount of paraffin to the bread samples as a fixative for the fatty acids.

Possibly it has not been emphasized that the filtercel pad must be dry before the extraction with petroleum ether is made, and no doubt this is the reason for low fat results by Analysts II and VI.

It seems to be quite generally agreed that it would be desirable to have another method for determination of non-fat milk solids in case the citric acid method needed confirmation. Therefore, since there have been two recent publications on the estimation of milk solids in bread based on the determination of lactose some study has been given these methods by the Associate Referee. These methods were published by D. A. Magraw and L. E. Copeland in *Cereal Chem.*, **13**, 541 (1936), and by C. Hoffman, T. R. Schweitzer, and G. Dalby in *Ind. Eng. Chem. Anal. Ed.*, **8**, 298 (1936).

In order to supplement and confirm the results of the Associate Referee by these two methods the crumb from loaves of the same composition as Samples 1, 2, and 3, referred to above, were sent to Hoffmann, Schweitzer and Dalby, and to Thompson, Magraw, and Copeland, and their cooperation was requested in the determination of milk solids by their respective methods.

Results by Hoffmann Method

SAMPLE NO.	H_2O	WHOLE MILK SOLIDS MOISTURE-FREE BASIS	WHOLE MILK SOLIDS PRESENT
	per cent	*per cent*	*per cent*
1	11.0	2.62	0
2	10.9	6.13	3.9
3	9.9	9.88	7.7

It will be noted that the results by the Hoffmann method are much too high.

Results by Thompson Method

SAMPLE NO.	LACTOSE M.F.B.	MILK SOLIDS NON-FAT MOISTURE-FREE BASIS	WHOLE MILK* SOLIDS, M.F.B.	MILK SOLIDS PRESENT
	per cent	*per cent*	*per cent*	*per cent*
1	0.34	0.68	0.96	0
2	1.78	3.58	5.05	
	1.79	3.79	5.14	3.9
			Av. 5.10	
3	3.02	6.09	8.59	
	3.04	6.13	8.65	
			Av. 8.62	7.7

* Calculated by Associate Referee from milk solids non-fat.

Comment by analysts: "The amount of lactose found in sample No. 1 was so very small that we question the accuracy of this result."

The results by the Thompson method are also too high, but they are nearer the true value. It is apparent that the results by these two methods are too high owing to the high value on the blank, namely, the water bread sample No. 1. Correction for the blank gives values very close to the true value for Samples No. 2 and No. 3 for both methods. However, at the present time such correction is not legitimate for general application. More information or further modification is necessary before a collaborative study of these methods should be made.

To the following collaborators, who generously cooperated in this study, many thanks are due and hereby given.

E. C. Thompson and associates, The Borden Co., New York City.
C. Hoffmann and associates, Ward Baking Co., New York City.
F. B. Jones, Food and Drug Administration, New York City.
J. H. Bornman, Food and Drug Administration, Chicago.
E. M. Bailey and D. C. Walden, Connecticut Agr. Exp. Sta., New Haven.
W. Catesby Jones and associates, Dept. Agr. & Immigration, Richmond.

RECOMMENDATIONS[1]

It is recommended—

(1) That the citric acid method be further studied collaboratively.

(2) That the fat method as outlined be studied with a view to modification before more collaborative work is done.

(3) That a study of the lactose methods be continued with a view to collaborative work should the facts warrant.

REPORT ON VISCOSITY OF FLOUR

STANDARDIZATION OF THE MACMICHAEL VISCOSIMETER

By C. G. HARREL (Pillsbury Flour Mills Co., Minneapolis, Minn.), *Associate Referee*

The values obtained by the Associate Referee by the use of the MacMichael viscosimeter are not true viscosity values or true plasticity values, but they represent a measurement of a combination of the two, that is viscosity and plasticity. This point has been discussed to some extent by Paul F. Sharp.[2] Other references that tend to bear this fact out are furnished by E. C. Bingham.[3] It is for this reason that the term "apparent viscosity" will be used throughout this report instead of the term "viscosity."

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[1] For report of Subcommittee D and action of this Association, see *This Journal*, 20, 66 (1937).
[2] *Cereal Chem.*, 3, 140 (1926).
[3] Fluidity and Plasticity, McGraw Hill Co., New York (1922); Plasticity—Colloid Control—Colloid Symposium Monograph, Vol. 2, 106 (1925).

Some collaborative work on making flour-water suspensions for apparent viscosity determinations is considered, and there is also pointed out through experimental work some of the places where errors may arise in apparent viscosity determinations; that is, the effect of moisture content of the flour, strength of the lactic acid, and bowl speed of the viscosimeter. Finally, there is presented for adoption as official a method of determining the apparent viscosity of acidulated flour-water suspensions.

COLLABORATIVE RESULTS

It was the purpose of this collaborative work to determine which of the three following methods of preparing the flour-water suspension for the apparent viscosity determination is the best. Two samples of flour labeled "A" and "B" were used.

The following instructions were sent to the collaborators:

Collaborative Work on Apparent Viscosity

The three following methods of preparing the flour-water suspension for the apparent viscosity test are to be carried out:

Method No. 1.—Suggested by Bayfield.

Place 20 grams of flour on a 15% moisture basis in a 500 cc. dry Erlenmeyer flask, add 100 cc. of distilled water at 30° C., and stopper the flask with a rubber stopper. Shake the flask vigorously for 1 minute. Remove the stopper and add 3 or 4 drops of caprylic alcohol. Replace the stopper and shake the flask 10 times to remove any foam. Pour the contents of the flask in the viscosimeter bowl and determine the apparent viscosity according to the method presented later in this report.

Method No. 2.—Described previously by C. G. Harrel in *This Journal*, **18**, 577 (1935); **19**, 555 (1936). This method utilizes a mortar and pestle of the following size:

(a) *Mortar size:*

Inside top diameter	9.5 cm.
Depth	5.5 cm.
Volume	300.0 cc.

(b) *Pestle size:* Diameter of butt—3.5 cm.

20 grams of flour on a 15% moisture basis is used.

Method No. 3.—Use a 200 cc. dye beaker and a half-inch glass stirring rod. Place 50 cc. of distilled water in the beaker, and place 20 grams of flour on a 15% moisture basis on top of the water. Mix this suspension with the glass stirring rod for 1½ minutes, then add 2 or 3 drops of caprylic alcohol and another 50 cc. of distilled water. Stir this suspension for about 3 seconds to insure uniform suspension, and then place the contents in the viscosimeter bowl. Determine the apparent viscosity according to the following method:

1. *Setting of Machine*—

   (a) *No. 30 wire.*

   (b) *Disc diameter.*—2.375", plus or minus 0.01".

   (c) *Disc thickness.*—0.25", plus or minus 0.01".

   (d) *Disc clearance.*—0.25".

TABLE 1.—*Sample A*

VOLUME OF ACID	METHOD 1					METHOD 2					METHOD 3				
	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
COLLABORATOR															
1	5	26	39	43	43	4	27	38	41	41	4	23	36	38	38
	5	26	38	42	42	4	24	36	39	39	4	24	35	38	38
2	7	30	41	44	44	6	29	41	42	43	5	32	44	46	47
	6	32	42	43	44	6	31	43	45	45	5	35	45	47	47
3	5	26	37	40	40	4	30	36	36	37	6	30	41	44	44
	3	20	35	38	40	5	30	41	44	45	7	32	42	43	43
4	7	26	37	40	40	5	25	36	38	39	5	26	36	39	40
	7	26	38	40	41	5	26	37	40	41	5	27	37	39	40
5	10	32	42	45	45	5	29	40	43	43	5	29	38	40	40
	9	32	42	44	44	5	30	40	43	43	5	29	39	40	40
6	4	27	38	40	40	4	29	36	39	40	4	27	37	39	40
	4	27	37	40	40	4	28	36	39	40	4	27	37	38	39
7	8	23	39	41	42	5	29	40	42	42	6	24	37	40	41
	7	24	40	41	42	6	30	40	42	42	6	24	36	39	40
8	6	30	40	42	42	4	31	42	46	47	4	31	43	46	46
	6	29	40	43	43	5	32	43	45	46	5	30	42	45	45
9	6	17	25	27	28	5	19	26	28	29	5	21	30	34	34
	5	17	26	27	29	4	22	31	34	33	4	22	32	35	35
10	5	23	33	36	37	4	23	34	36	39	4	23	34	37	38
	6	25	35	37	38	4	25	36	38	39	4	24	36	40	41
11	15	32	44	48	49	4	20	29	36	38	3	23	29	35	37
Average	6.52	26.2	37.5	40 0	40.6	4.66	27.1	37.1	39.7	40.5	4.76	26.7	37.4	40.1	40.6

TABLE 2.—*Sample B*

	METHOD 1					METHOD 2					METHOD 3				
VOLUME OF ACID	0	1	3	5	7	0	1	3	5	7	0	1	3	5	7
COLLABORATOR															
1	20	52	98	107	107	12	52	98	110	112	12	48	91	101	104
	18	53	96	105	105	11	51	102	114	114	10	47	91	101	103
2	18	54	111	120	120	15	45	112	122	123	12	52	105	117	119
	17	53	110	119	120	16	55	110	121	122	10	54	109	123	127
3	21	51	100	114	115	18	61	108	118	123	13	48	92	98	101
	22	65	120	128	125	13	57	106	115	115	8	50	92	107	105
4	18	45	93	106	109	11	40	93	104	107	11	37	85	98	101
	20	45	94	105	108	11	39	93	104	107	11	39	89	101	105
5	19	64	109	118	119	15	63	113	120	122	13	54	101	114	116
	20	64	109	118	119	16	63	109	120	122	13	56	102	114	116
6	18	53	88	97	99	12	59	102	112	112	11	57	93	99	104
	18	51	88	99	99	10	54	101	111	113	10	59	95	103	105
7	17	49	104	116	117	13	48	102	114	116	13	44	97	111	113
	18	55	108	120	121	13	48	103	114	117	13	44	97	112	113
8	14	51	103	107	107	10	46	99	110	110	10	42	94	106	110
	14	47	95	105	106	11	44	94	107	109	10	37	91	102	106
9	15	36	73	83	83	10	42	90	107	110	11	36	84	99	103
	15	36	75	83	83	9	38	82	97	100	10	38	85	99	103
10	17	40	91	102	103	10	37	92	104	106	10	32	92	105	106
	17	39	89	101	104	9	36	91	106	109	10	35	91	104	105
11	22	59	65	105	108	10	40	95	106	110	9	45	90	103	105
Average	18	50.6	96.2	107.4	108.3	12.1	48.4	99.7	111.1	113.2	10.95	45.4	93.6	105.5	108

(e) *Viscosimeter bowl.*—Diameter, 2.75", plus or minus 0.01". The bowl should not be dented or out of shape.
(f) *Speed.*—Bowl should make 12 revolutions per minute.
(g) *Empty machine.*—Adjust for zero reading.

RUNNING APPARENT VISCOSITY

After placing the flour-water suspension in the bowl, stir it up and down with the disc about 15 times. Start the motor, taking the reading of the apparent viscosity in degrees MacMichael. When the disc starts to swing, dampen the swing by placing the tip of the finger on the viscosimeter pointer and gradually lower the hand so that part of the finger comes in contact with the swinging dial. When the dial comes to rest (after dampening), take the reading.

Without stopping the motor, add 1 cc. of 1 *N* lactic acid; stir the suspension with the disc and again take the apparent viscosity reading. Take these readings with 2 cc. increments of 1 *N* lactic acid until a total of 9 cc. has been added, stirring the suspension with the disc after each addition of the acid.

Run duplicate results and report on the Data Sheet furnished.

Sample A has a moisture content of 10.4; therefore use an 18.96 gram sample. Sample B has a moisture content of 12.5; therefore use a 19.42 gram sample.

RESULTS OF COLLABORATIVE WORK

Tables 1 and 2 give the results of the collaborators on apparent viscosity. Also at the bottom of each table are given the average results.

Table 3 shows the standard deviation and the per cent standard deviation of the apparent viscosity results according to the three methods of preparing the flour-water suspension.

TABLE 3.—*Standard deviation*

METHOD OF PREPARING SUSPENSION—	SAMPLE A	SAMPLE B	SAMPLE A	SAMPLE B	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1 (shaking in flask)	4.8	12.3	11.8	10.4	11.1
2 (mortar & pestle)	3.87	7.65	95.5	6.76	8.15
3 (stirring rod & beaker)	3.74	9.69	9 22	8.97	9.09

It may be noted in Table 2 that Method 2 gives a slightly higher apparent viscosity result than either of the other two methods presented, indicating that this method of preparation causes a slight development of the gluten.

As shown in Table 3 Method 1 gives the largest per cent deviation, Method 3 gives the next largest, and Method 2 the smallest per cent deviation. However, the difference is not exceedingly great, and it may be caused by the fact that the collaborators were not accustomed to these methods of preparing flour-water suspensions.

From Tables 1 and 2, it is evident that the collaborators get better checks upon duplicate determinations when using Method 1. Therefore

it is believed that because of the ease and simplicity of preparing the suspension by Method 1, and because it produces better duplicate results, it should be adopted as the official method of preparing flour-water suspensions for apparent viscosity determinations.

EXPERIMENTAL WORK

It is known that lactic acid solutions that are not properly made increase in strength upon standing. Three solutions of lactic acid of different normalities (.788, 1.002, and 1.229) were prepared, and apparent viscosities were determined on two different flours by means of these three different strength acids. The results, Table 4, are the average of three determinations.

TABLE 4.—*Apparent viscosities*

Sample C, moisture 10.5%, 18.98 gram sample used

VOL. OF ACID USED	0	1	3	5	7
Acid normality .788	8	49	77	84	84
Acid normality 1.002	8	55	81	85	85
Acid normality 1.229	8	60	84	88	88
Sample D, moisture 12.1%, 19.33 gram sample used					
Acid normality .788	5	22	30	32	32
Acid normality 1.002	5	23	32	33	33
Acid normality 1.229	5	25	33	34	34

The effect of the strength of acid used is greatest, as would be expected, when the first cc. is introduced into the flour-water suspension. Also (Table 4) the strength of acid will influence the type of curve when volume of acid is plotted against apparent viscosity. If the curve is to be plotted, the acid should be carefully prepared and standardized. If only the final reading is to be considered, the normality of tle acid is not quite so important, although the reading is slightly higher with the higher normality acids.

The next investigation was in regard to moisture content of the flour. Samples C and D were also used for this work. Sample C had a moisture content of 10.5 per cent, and D one of 12.1 per cent. Apparent viscosities were run on the two flours and the moisture content was assumed to be 1 per cent above the actual moisture and 1 per cent below the actual moisture when corrected to 15 per cent moisture basis. The results (Table 5) are the average of three determinations.

TABLE 5.—*Moisture content*

Sample C, Moisture 10.5%

VOL. OF ACID	0	1	3	5	7
Assuming 9.5% Moisture 18.78 grams used	8	55	80	84	84
Actual 10.5% Moisture 18.98 grams used	8	55	81	85	85
Assuming 11.5% Moisture 19.2 grams used	8	56	81	86	86
Sample D, Moisture 12.1%					
Assuming 11.1% Moisture 19.1 grams used	5	24	31	32	32
Actual 12.1% Moisture 19.33 grams used	5	24	32	33	33
Assuming 13.1% Moisture 19.56 grams used	5	24	32	33	33

In Table 5 it is seen that an error of plus or minus 1 per cent in the moisture content of the flour has little effect upon the apparent viscosity results, and therefore it can be assumed that few errors are caused by incorrect moisture determinations.

The last piece of experimental work was to determine the effect on the apparent viscosity of a plus or minus 2 r.p.m. variation in bowl speed at 12 r.p.m. Flour samples C and D were used, and the results (Table 6) are the average of three determinations.

TABLE 6.—*Effect of bowl speed*

VOL. OF ACID USED	0	1	3	5	7	0	1	3	5	7
	Sample C					Sample D				
r.p.m.										
Bowl Speed 10	7	51	75	77	77	4	21	29	30	30
Bowl Speed 12	8	55	81	85	85	5	24	32	33	33
Bowl Speed 14	9	60	87	93	93	6	27	35	36	36

From Table 6 it can be seen that a variation in bowl speed does not make a great change in the type of curve (plotting apparent viscosity against volume of acid). However, the variation of 1 r.p.m. causes a variation of about 4.6 per cent of the final apparent viscosity. It may be possible that incorrect bowl speeds are contributing to inconsistent results among collaborators.

CONCLUSIONS AND RECOMMENDATIONS[1]

(1) That Method 1 (shaking the flour and water together in a 500 cc. Erlenmeyer flask as suggested by Bayfield) be adopted as the standard

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 66 (1937).

method of preparing the flour-water suspension for the apparent viscosity determination.

(2) That as the normality of the lactic acid has a marked effect upon the apparent viscosity it should be carefully prepared and standardized.

(3) That an error of plus or minus 1 per cent in determining the moisture content of flour has little effect upon the apparent viscosity.

(4) That since a variation of 1 r.p.m. in bowl speed has a marked effect on apparent viscosity a stop-watch in checking this speed should be used.

COLLABORATORS

Pillsbury Flour Mills Co., Minneapolis, Minn.
Pillsbury Flour Mills Co., Astoria, Ore.
Kansas Flour Mills, W. L. Heald, Kansas City, Mo.
The W. E. Long Co., Chicago, Ill.
F. W. Stocks & Son, Sleeper & Rosenberry, Hillsdale, Mich.
Mid West Laboratories, H. M. Simmons, Columbus, Ohio
Igleheart Bros., Inc., Evansville, Ind.
Kroger Food Foundation, G. Garnatz, Cincinnati, Ohio
Pillsbury Flour Mills Co., Springfield, Ill.
United Mills Co., W. E. Brownlee, Grafton, Ohio
Technical Institute of The Independent Biscuit Mfg. Co., R. M. Bohn and D. E. Kinder, Chicago, Ill.

The following method is presented for adoption as official for the determination of apparent viscosity of acidulated flour-water suspension:

APPARENT VISCOSITY OF ACIDULATED FLOUR-WATER SUSPENSION BY MACMICHAEL VISCOSIMETER

ADJUSTMENT OF MACHINE

(a) Use a No. 30 MacMichael viscosimeter wire.

(b) Have the diameter of the disc plunger 2.375", plus or minus 0.01".

(c) Adjust the machine so that the clearance between the bottom of the disc and the inner surface of the bottom of the bowl is 0.25", plus or minus 0.005". Check this clearance carefully with a depth gage reading in 0.001".

(d) Use a viscosimeter bowl having a diameter of approximately 7 cm. (The depth of the bowl will vary according to the age of the machine.)

(e) Adjust the regulating device to permit a speed of exactly 12 r.p.m., and check it very carefully and frequently with a stop-watch, because as the motor warms up the machine will have a tendency to run faster.

(f) Adjust the machine and keep it level, and when the bob is placed see that it is riding freely and is not touching the sides of the guide.

(g) Adjust the dial so that when it comes to rest the pointer is on the zero mark.

PREPARATION OF LACTIC ACID

Add to the concentrated lactic acid approximately the right proportion of water to give a slightly stronger solution than normal. Reflux this solution 3 hours, cool, and by the addition of water adjust to normal. It may also be prepared as directed by Reiman[1] as follows: Use enough concentrated lactic acid to prepare a solution approximately 0.8500 N when standardized with 0.1 N NaOH. Transfer this solution to an Erlenmeyer flask, fitted with an air condenser to prevent undue evapora-

[1] *Cereal Chem.*, 11, 299 (1934).

tion of water, and heat at a temperature of 80° C. for 24 hours. (The solution will have increased in strength to 1.183 *N*.) Add enough distilled water to bring the solution to exactly normal.

PREPARATION OF FLOUR-WATER SUSPENSION

Into a clean, dry, 500 cc. Erlenmeyer flask, place 20 grams of flour on 15% moisture basis and add 100 cc. of distilled water at 30° C. Place a rubber stopper in the mouth of the flask and shake vigorously for 1 minute. Place the flask in a constant temperature cabinet or water bath at 30° C. for a period of 1 hour, shaking about 10 times every 15 minutes. Remove the flask from the cabinet or water bath, add 3 or 4 drops of caprylic alcohol, shake 10 times to remove any foam which may be present, and pour the suspension in the bowl of the viscosimeter.

DETERMINATION

After pouring the suspension into the viscosimeter bowl, make sure that the bowl is down flush upon its supports. Start the machine, but before placing the bob or disc in place, stir the solution with the bob 25 times to insure a uniform suspension. Place the wire of the bob in its holder and take the reading after dampening the swing of the dial by placing a finger on the indicator pointer and then gradually touching the swinging dial. Make the second reading after the addition of 1 cc. of normal lactic acid, and likewise the third and following readings after the addition of 2 cc. increments of the normal lactic acid. Do not stop the motor between readings. After or during the addition of lactic acid, stir the suspension 25 times by an up and down motion of the bob. Suspend the bob by the wire and take the reading. Determine the maximum apparent viscosity of the acidulated flour-water suspension by plotting the apparent viscosity readings against the volume of acid added. Usually a total of 7 cc. of 1 *N* lactic acid is sufficient to give the maximum reading, but 2 cc. increments should be added continuously until no further increase in apparent viscosity is noted.

DISCUSSION

It is noted that in the method proposed for adoption as official, a 1 hour digestion period for the flour-water suspension is to be used. This digestion period will give better checks than a no-time method. However, the work described in this report was done on the no-time method, and fairly good results were obtained. For control purposes a no-time method could be used; however, there is no relationship between the results obtained by the no-time method and the one hour digestion method, and the results of the two methods cannot be used for comparison.

If the character of the gluten is to be studied, the method proposed for adoption can be used, and instead of using 20 grams of flour on the 15 per cent moisture basis, the weight of the flour can be taken on the protein basis.

ACKNOWLEDGMENT

This report was made possible through the courteous and prompt support of the collaborators, and due credit is hereby given them. Walter Tholstrup presented many valuable suggestions and conducted the determinations presented in this report and also supervised the collaborative work. To him heartiest appreciation is extended.

No report on cold water extract flour was given by the associate referee.

No report on ergot in flour was given by the associate referee.

No report on catalase and proteolytic enzymes was given by the associate referee.

REPORT ON COLOR IN FLOUR

By H. K. PARKER (Wallace and Tiernan Co., Inc., Newark, N. J.), *Associate Referee*

Further collaborative tests with the N-A Colorimeter were carried out to try to determine differences between observers, as well as to study how closely their results checked on the same flours. These tests were conducted as nearly as possible under controlled conditions of light intensity, avoidance of side lights, and uniform wetting and drying time. The same collaborators mentioned in last year's report, *This Journal*, **19**, 569 (1936), participated.

For test No. 5 three samples were sent out in sealed Mason jars, and as soon as possible after new discs had been supplied to collaborators. The samples were designated A, B, and C; A and C were identically the same flour but this fact was unknown to the various observers.

The results are recorded in Table 1.

The agreement between observers is not especially close, and corresponds to the findings of Tests 3 and 4 reported last year. It is interesting to note, however, that the average of individual average readings for flour A compares very well with the average of average readings for flour C.

Test 6 was carried out some weeks later. Flour B of Test 5 was refrigerated and kept in the dark until sent out for this test. Thus it was possible to compare again the readings of different observers upon the same flour. The test corresponds to collaborative Tests 3 and 4 reported last year.

The results are recorded in Table 2.

It is to be noted that different values are obtained by the various observers, and many times even in the same laboratory.

In order to study these results, it is interesting to assume an arbitrary limit as to the observers' ability to check themselves upon the same flours. If one places a limit of 1 point upon the average yellow reading, which has a value of 24; a limit of 0.33 of a point upon the average red reading, which has a value of 8; a limit of 0.15 of a point upon the average black reading, which has a value of 3.5; and a limit of 2.6 points upon the

TABLE 1.—*Collaborative test No. 5*

		FLOUR A				FLOUR B				FLOUR C			
OBSERVER NO.	LAB.	Y	R	B	W	Y	R	B	W	Y	R	B	W
1	A	24.76	7.64	2.88	64.72	19.60	7.44	3.50	69.46	24.38	7.46	2.84	65.32
2	B	25.3	8.1	4.3	62.3	18.9	8 7	4.7	67.7	24.8	8.4	3.9	62.9
3	B	26.7	7.5	4.0	61.8	21.3	7.2	3.8	67.7	27.2	6.2	3.6	63.0
4	A	—	—	—	—	—	—	—	—	—	—	—	—
5	C	25.8	10.3	2.3	61.6	22.0	9.2	3.0	65.8	23.3	10.0	2.3	64.4
6	C	23.4	10.0	2.8	63.8	22.9	9.1	3.4	64.6	23.5	9.9	2.9	64.7
7	D	21.2	9.2	3.3	66.3	18.8	9.6	3.1	68.5	23.8	9.1	3.2	63.9
8	D	19.7	9.5	3.5	67.3	17.8	10.1	3.3	68.8	23.5	9.5	3.7	63.3
9	M	26.2	5.86	4.5	63.38	19.58	6.5	4.49	69.6	24.88	6.12	4.12	64.86
10	F	25.2	8.0	4.2	62.6	21.9	7.8	3.5	66.9	26.8	8.0	2.9	62.30
11	G	27.80	7.50	2.60	62.10	23.5	7.70	2.50	66.30	28.00	7.60	2.10	62.30
12	H	23.48	9.21	8.60	58.71	21.5	9.3	6.0	63.2	20.81	9.27	6.90	63.02
13	H	24.4	7.6	7.2	60.8	20.9	6.7	8.8	63.6	25.6	6.7	7.3	60.4
14	G	25.34	8.44	1.18	65.04	22.42	8.16	1.56	67.86	27.56	7.78	1.32	63.34
15	E	27.42	6.34	4.66	61.38	21.92	7.18	4.92	65.98	27.85	6.68	4.44	61.04
16	C	22.5	9.8	3.1	64.6	21.2	8.0	4.5	66.3	23.5	8.6	3.4	64.5
17		—	—	—	—	—	—	—	—	—	—	—	—
18	A	25.8	6.8	2.6	64.8	19.1	7.5	2.9	70.5	26.4	6.9	2.6	64.1
Average of Averages		24.7	8.24	3.86	63.2	20.83	8.14	3.99	67.05	25.12	8.01	3.59	63.3

TABLE 2.—*Collaborative test No. 6*

OBSERVER NO.	LAB.	TEST 6* Y	R	B	W	TEST 5—FLOUR B Y	R	B	W
1	A	19.53	7.01	2.82	70.64	19.6	7.44	3.50	69.46
18	A	18.12	6.83	3.43	71.62	19.1	7.5	2.9	70.5
2	B	20.50	7.70	4.20	67.60	18.9	8.7	4.7	67.7
3	B	20.00	9.20	2.90	67.90	21.3	7.2	3.8	67.7
5	C	22.05	7.50	3.60	66.85	22.0	9.2	3.0	65.8
6	C	19.80	9.50	3.60	67.10	22.9	9.1	3.4	64.6
16	C	19.85	8.45	3.90	67.80	21.2	8.0	4.5	66.3
7	D	18.92	7.89	3.52	69.67	18.8	9.6	3.1	68.5
8	D	19.75	7.67	4.01	68.57	17.8	10.1	3.3	68.8
9	M					19.58	6.5	4.49	69.6
10	F	18.30	10.60	2.10	69.00	21.9	7.8	3.5	66.9
11	G	22.48	9.43	2.43	65.98	22.42	8.16	1.56	67.80
12	H	20.39	6.98	8.36	64.40	25.6	6.7	7.3	60.4
13	H					20.81	9.27	6.9	63.02
14	G	21.50	8.29	2.07	68.14	23.50	7.70	2.5	66.30
15	E	21.84	7.63	5.77	64.76	21.92	7.18	4.92	65.98
13	H	22.93	10.08	6.09	60.93				
Average of Averages		20.39	8.31	3.92	67.40	20.83	8.14	3.99	67.05

* Flour same as B in Test 5 (refrigerated between tests).

average white reading, which has a value of 63, then a comparison can be made as in Table 3.

TABLE 3.—*Percentage of observers within arbitrary limit*

COLOR	LIMIT OF READING	TESTS 3 & 4	TEST 5 (A & C)	TEST 6
		per cent	*per cent*	*per cent*
Yellow	1.0	60.0	50.0	50.0
Red	0.33	13.3	75.0	7.1
Black	0.15	13.3	43.7	0.0
White	2.6	80.4	81.0	92.8
White	1.0	20.0	56.2	35.0

When these figures are tabulated it appears evident that no real advance in reading ability has been made as the tests have progressed. The percentage of observers within the limit for the yellow and white is fairly satisfactory, and possibly indicates that these readings are easier to duplicate than the red or black. It must be admitted, of course, that since these are saturated colors of lower reflectivity, the arbitrary limit imposed may be much too small. It is difficult to speculate as to the reason for the better red and black results when the same flour was determined on the

same day as in Test 5. It is possible that more uniform conditions prevailed, during wetting and drying the wet slick, than occurred in such tests as Nos. 3, 4, and 6.

There is definite evidence that the instrument should be placed in a dark room for most accurate results and to assure that outside light does not affect the black reading and the readings as a whole. It has been noted that readings change with out-of-door light intensity variation even though the determinations are made in a room darkened by drawn shades. Thus it may be explained that the outside light was more uniform during Test 5 on one single day than was the outside light on different days (Tests 3, 4, and 6).

The 1 point white limit was added to the table out of curiosity to learn what accuracy could be expected for brilliancy.

A classification of observers indicates that only about 35 per cent will be within the above prescribed limits with 50 per cent of their readings; 65 per cent will have less than 50 per cent within limits.

Concerning the other recommendations made last year, it is regretted that no progress can be reported. While the Maxwell disc type of colorimeter certainly has its uses as a research tool, there is no indication from the collaborators, after two years' trial, that it is interesting or practical to use as a routine measurement instrument for flour color in mill or bakery. The main objections seem to be that the determination is time consuming, and somewhat tiring to the eye. These together with the facts stated above concerning the discrepancies between observers discourage recommendation as to further work which might lead to this means for routine flour color measurement or specification, even though results to date indicate that this method of color determination seems the most satisfactory yet developed.

Apparently it is much easier and more rapid to compare 50 flours against a given standard by Pekarization, than to use the instrument on each. It is interesting to hear, however, that certain collaborators are using the machine to keep their standard flour color from drifting; that is to say, new standards are prepared and blended by using the figures of the N-A Colorimeter. It is known, of course, that the color of standard flours becomes lighter with age, so certainly this method of color evaluation should be of some importance for mill or bakery control along these lines—a reproducible standard flour for Pekarization against the "run of the mill."

It has been suggested that the instrument is also of value in classifying observers for it definitely shows those outside the normal.

While at this stage it is difficult to make recommendations[1] as to further work leading to establishment of flour color types and standards, it is yet possible that improvement of technic, more experience, and better selec-

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 66 (1937).

tion and training of eyes may lead to interesting results. Perhaps one should not be too discouraged if results of the first collaborative protein determinations be examined critically. Before collaborative work is dropped, it is recommended that comparative cooperative readings be made by using the machines in dark rooms, or under some specified cover, so that no possible side light or change of outside light intensity can interfere.

TUESDAY—AFTERNOON SESSION

REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration, Chicago, Ill.), *Referee*

Work on standard solutions this year was limited to a study of well-known methods for standardizing solutions of 0.1 *N* sulfuric acid.

Direct standardization methods are to be preferred generally, and work thus far has dealt with this type of determination. A procedure that offers promise is that based on the volatilization of ammonium sulfate. The Referee has made a study of this method, but the results have not warranted collaborative study.

METHOD[1]

PREPARATION OF APPROXIMATELY 0.1 *N* SULFURIC ACID

Add 29 cc. of concentrated H_2SO_4 to 10 liters of H_2O and mix.

STANDARDIZATION

Pipet 50 ml. of the 0.1 *N* acid into a Pt dish that has been heated to constant weight at 600°C. Place the dish containing the acid in a bell jar with about 100 cc. of ammonia in a 250 cc. beaker for at least 14 hours. Evaporate the solution in the Pt dish to dryness on a steam bath and dry for 4 hours to constant weight in an electric oven at 106°C. Volatilize the $(NH_4)_2SO_4$ at 600°C. in an electric muffle, heating until the weight of residue, if any in the Pt dish, is constant (1 hour is generally sufficient). Weight of $(NH_4)_2SO_4 \times 0.30270$ = normality of the solution.

Table 1 shows the results of this procedure compared with the normality obtained by checking against 0.1 *N* NaOH and using phenolphthalein as an indicator.

TABLE 1

$(NH_4)_2SO_4$	$HKC_8H_4O_4-NaOH-H_2SO_4$
0.10022	0.10040
0.10034	0.10042
0.10013	0.10046
0.10019	
0.10022	Av. 0.10043 *N*
0.10025	
Av. 0.10023 *N*	

DISCUSSION

There is no doubt but that the standard solution should be more nearly accurate than that of the analysis for which it is needed, since such errors of standardization may be added to the final result. The range of the above normality values is two parts in a thousand. Obviously the

[1] Modification of method reported in Scott's Standard Methods of Chemical Analysis, Vol. 11, p. 1495. D. Van Nostrand Co. This procedure does not volatilize the $(NH_4)_2SO_4$.

ammonium sulfate procedure is not sufficiently precise for use in exact volumetric analysis. However, the Referee believes that further study of this method is warranted.

It is recommended[1] that studies of methods of direct standardization of acid solutions be continued, and that standard solutions of oxidation and reduction be studied.

REPORT ON INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

By J. J. T. GRAHAM (Insecticide Division, U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The work for 1936 consisted of the study of methods for the determination of rotenone in derris and cube powder, and the pyrethrins in pyrethrum powder. Collaborative work was done only on the pyrethrum powder.

PYRETHRUM POWDER

As it was believed to be advisable to have a comparison made between the results on old and new powders, Sample 1 was taken from a lot of powder purchased in 1932, while the other samples were bought on the 1936 market. These samples were sent to the collaborators with the following directions for analysis:

PYRETHRINS IN PYRETHRUM POWDER

Pyrethrin I[2]

REAGENTS

(a) *Alcoholic sodium hydroxide solution.*—Approximately 0.5 *N*.
(b) *Standard sodium hydroxide solution.*—0.02 *N*.
(c) *Barium chloride.*—10% solution.
(d) *Petroleum ether.*—Distillation range 30–60°.
(e) *Filter-cel.*

DETERMINATION

Extract 12.5 grams of flowers ground to 30 mesh, or finer, in a Soxhlet extractor for 7 hours with petroleum ether. Evaporate the ether on a water bath, heating no longer than necessary to remove all the solvent. Add 15 cc. of the alcoholic NaOH solution to the residue and boil gently under a reflux condenser for 1.5–2 hours. Transfer the alkaline alcoholic solution to a 600 cc. beaker, washing the flask with water. Add sufficient water to bring the volume of liquid in the beaker to 200 cc. Add a few glass beads or introduce a boiling tube and boil to remove the alcohol, using care to avoid boiling over, due to saponification. When the volume has been reduced to 150 cc., cool and transfer to a 250 cc. volumetric flask to which has been added 1 gram of filter-cel. Thoroughly mix the solution to distribute the filter-cel, add 10 cc. of the $BaCl_2$ solution and make to volume. Mix the solution by thorough shaking and after the precipitate has settled, filter through a fluted paper. Transfer 200 cc. of the clear filtrate to a 500 cc. flask, add 5 cc. of H_2SO_4 (1+4) and distil with steam, using a distillation trap and an efficient condenser. Receive the distillate in a 500 cc.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 48 (1937).
[2] Seil. *Soap*, 10, 89, 91, 111 (1934)

separatory funnel and continue the distillation until the volume of liquid in the flask is reduced to about 20 cc. The quantity in the separatory funnel should be about 250–350 cc. At this point the separatory funnel will contain the mono-carboxylic acid and the distillation flask the di-carboxylic acid.

To the separatory funnel add 50 cc. of petroleum ether and shake thoroughly for 1 minute. After the liquids have separated, draw off the aqueous layer into a second separatory funnel and again extract with 50 cc. of petroleum ether. Draw off the aqueous layer into a third funnel, extract with 25 cc. of petroleum ether, and discard the aqueous layer. Wash the petroleum ether extracts in the three funnels successively with 10 cc. of water and discard the water after it has passed through the third funnel. Wash with another 10 cc. of water and discard as before. Combine the petroleum ether extracts. Neutralize 15 cc. of water containing 1 or 2 drops of phenolphthalein indicator solution with 0.02 *N* NaOH solution, add it to the combined petroleum ether solutions, and titrate with 0.02 *N* NaOH solution, shaking after each addition, until the aqueous layer is just pink. Each cc. of 0.02 *N* NaOH solution consumed is equal to 0.0066 gram of Pyrethrin I. The 200 cc. aliquot part taken corresponds to 10 g. of sample, therefore the number of cc. of NaOH solution consumed $\times 0.066$ gives the percentage of Pyrethrin I.

Pyrethrin II[1]

Method 1

Allow the residue from the steam distillation to cool and filter through a Gooch crucible, washing the flask with a little water. Make the clear filtrate alkaline with $NaHCO_3$, transfer to a separatory funnel, and wash twice with $CHCl_3$. Wash the $CHCl_3$ extracts successively through one wash water of 10–15 cc. and combine the aqueous solutions. Acidify with about 10 cc. of HCl and saturate with salt, adding very cautiously at first to avoid excessive ebullition of CO_2. Extract with 50 cc. of ether, shaking for about 1 minute. Draw off the aqueous layer into a second funnel and again extract with 50 cc. of ether. Continue the extractions in a third and a fourth funnel, using in each case 35 cc. of ether. Each time that the aqueous solutions or washings are drawn off from the separatory funnels use care to see that the droplets adhering to the walls are removed as completely as possible. Wash the four ether extracts successively with two 10 cc. portions of water and then combine the ether solutions. Tap off any water that separates and filter the ether solution into a 500 cc. Erlenmeyer flask. Evaporate the ether on a water bath and dry the residue at 100° for 10 minutes. Add 2 cc. of neutral alcohol, warm gently, add 20 cc. of water, and heat to dissolve the acid. If a residue remains undissolved, cool, and filter through a Gooch crucible. Add 1 or 2 drops of phenolphthalein indicator solution and titrate with 0.02 *N* NaOH solution. 1 cc. of 0.02 *N* NaOH solution = 0.00374 gram of Pyrethrin II.

Method 2 (Methoxyl Method)[2]

REAGENTS

(a) *Hydriodic acid.*—Sp. gr. 1.70, constant boiling, which has been treated with H_3PO_2 to remove free I. To reduce the blank, it is desirable to pass a stream of CO_2 through the boiling solution under reflux for 2–3 hours. (The hydriodic acid furnished by Merck & Co. has been treated with H_3PO_2.)

(b) *Potassium acetate solution.*—Dissolve 20 grams of $KC_2H_3O_2$ in 200 cc. of glacial acetic acid.

(c) *Sodium acetate solution.*—Dissolve 25 grams of $NaC_2H_3O_2$ in 100 cc. of water.

(d) *Formic acid.*—At least 90% pure.

(e) *Sodium thiosulfate solution.*—0.05 *N*.

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[1] Seil. *Soap*, 10, 89, 91, 111 (1934).
[2] Haller and Acree, *Ind. Eng. Chem. Anal. Ed.*, 7, 343 (1935).

APPARATUS

The apparatus is described by Clark (*This Journal*, *15*, *138* (*1932*)). *It consists* of a boiling flask, A; a scrubber, B, containing a little water; and the receivers, C and D.

DETERMINATION

Extract 5 grams of flowers ground to 30 mesh, or finer, in a Soxhlet or other suitable extractor for 7 hours with petroleum ether (distillation range 30–60°). After the extraction is complete, evaporate the ether on a steam bath, heating no longer than necessary to remove the solvent. Transfer the residue to the flask of the methoxyl apparatus by means of 20 cc. of $CHCl_3$ used in small portions. Place a small boiling rod in the flask, evaporate the $CHCl_3$ by heating in a water bath, removing the last traces under reduced pressure. Add 2.5 cc. of melted analytical quality phenol and 5 cc. of the HI and connect the flask with the rest of the apparatus. Put into the absorption tubes 10 cc. of the $KC_2H_3O_2$ solution to which 20 drops of Br has been added. (Unless a large excess of Br is used, low results will be obtained.) Boil the mixture in the flasks at such a rate that the vapors do not rise more than half the length of the condenser, and at the same time pass a slow stream of CO_2 through the flask.

After 1.5 hours discontinue the boiling and wash the contents of the absorption tubes into a flask containing 5 cc. of the $NaC_2H_3O_2$ solution. Adjust the volume of liquid to about 125 cc., and add 20 drops of formic acid to remove the excess of Br. When the liquid has become water-white and the last traces of Br have been removed, add 2 cc. of H_2SO_4 (1+10) and follow with 1 gram of KI. Titrate the free I thus liberated with 0.05 N $Na_2S_2O_3$ solution. 1 cc. of the thiosulfate solution is equivalent to 3.11 mg. of Pyrethrin II. From this value and the number of cc. used for titration, corrected for a blank determination using all reagents, calculate the percentage of Pyrethrin II in the sample.

The results (Table 1) show that apparently the analysts had no difficulty in reproducing their own determinations when they used the Seil method, but that there is a lack of uniformity among the results reported by the different analysts.

Wilcoxon[1] has shown that in the Seil method as much as 25 per cent of the monocarboxylic acid fails to pass over during the steam distillation. This error is partly balanced by the titration of other volatile acids along with the monocarboxylic acid, but the results are always below the correct values. The fact that not all the monocarboxylic acid is distilled and that other volatile acids are distilled along with that part that does distil, probably accounts for the divergent results reported by the different analysts.

Only two of the collaborators reported results by the Haller-Acree method for Pyrethrin II. On Samples 2 and 3 these analysts have checked each other very well, but there is too wide a difference in their results on Sample 1. Haller and Acree had used this sample two years before in their study of the method, and at that time they obtained 0.38 per cent as an average of eight closely agreeing results.

Extracts of each of these samples were prepared in a highly refined mineral oil base and for actual killing power were tested on house flies

[1] Contributions from Boyce Thompson Institute, 8, No. 3, 175–181 (1936).

TABLE 1.—*Collaborative results on pyrethrum powder*
(Results expressed as percentages)

ANALYST	DATE OF ANALYSIS	SAMPLE 1				SAMPLE 2				SAMPLE 3			
		PYRETHRINS				PYRETHRINS				PYRETHRINS			
		SEIL METHOD			HALLER-ACREE METHOD	SEIL METHOD			HALLER-ACREE METHOD	SEIL METHOD			HALLER-ACREE METHOD
		I	II	TOTAL	II	I	II	TOTAL	II	I	II	TOTAL	II
A. J. Cox Sacramento, Calif.	July	.31	.33	.64	.27	.45	.50	.95	.47	.40	.46	.86	.42
J. J. T. Graham	Feb.	.32	.35	.67	—	.42	.60	1.02	—	.35	.52	.87	—
	March	.30	.35	.65	—	.41	.59	1.00	—	.35	.51	.86	—
Average		.31	.35	.66		.42	.59	1.01		.35	.52	.87	
	Oct.	—	—	—	.36	—	—	—	.49	—	—	—	.39
		—	—	—	.38	—	—	—	—	—	—	—	—
Average					.37								
M. S. Lowman Washington, D. C.	Oct.	.22	.28	.50	—	.23	.48	.71	—	.19	.41	.60	—
	May	.24	.26	.50	—	.24	.48	.72	—	.22	.42	.64	—
	May	.25	.28	.53	—	—	—	—	—	—	—	—	—
Average		.24	.27	.51		.24	.48	.72		.21	.41	.62	
R. D. Stanley Chicago, Ill.	July	.26	.30	.56	—	.35	.43	.78	—	.28	.44	.72	—
	Aug.	.26	.31	.57	—	.34	.42	.76	—	.28	.44	.72	—
		.29	.30	.59	—	—	—	—	—	—	—	—	—
Average		.27	.30	.57		.35	.42	.77		.28	.44	.72	
Harry Bois San Francisco, Calif.	Sept. and Nov.	.29	.39	.68	—	.36	.49	.85	—	.33	.42	.75	—
		.28	—	—	—	.36	.52	.88	—	.33	.42	.75	—
Average		.29	.39	.68	—	.36	.51	.87	—	.33	.42	.75	—

by S. C. Billings at the Insecticide Testing Station of the Food and Drug Administration. The room test method was used, and the extracts were diluted so as to contain 0.05 per cent of total pyrethrins, based on the analytical results of the referee. The following results are the averages of 10 determinations:

SAMPLE	FLIES PARALYZED	FLIES DEAD
	per cent	*per cent*
1	83.8	62.1
2	92.0	71.9
3	94.3	78.0

If the analytical method had given correct results the percentages of flies killed by the three solutions should have been the same since the solutions were diluted to the same pyrethrin content. As stated previously, Sample 1 was purchased four years before the others, and it may be assumed to be that much older. It is to be noted that this sample gave the lowest percentage of kill of any of the samples. This fact and similar results from other tests (unpublished), suggest that pyrethrins in older samples may undergo some change that makes them of less insecticidal value than the pyrethrins from new pyrethrum.

DERRIS AND CUBE POWDER

The study of methods for the determination of rotenone did not reach the stage where it was desirable to ask for collaborative work. The Referee is investigating a number of methods for this determination, and they will probably be in shape to be submitted for collaborative study next year.

FUTURE WORK

Wilcoxon[1] has proposed a modification of the Seil method for Pyrethrin I, wherein he substitutes extraction for distillation as a means to separate the pyrethrin, after which he determines it by iodate titration of the mercurous salt obtained by the reduction of Denige's reagent with the monocarboxylic acid. His results by this modification were always higher than those obtained by the regular Seil method. This method should be included in the pyrethrum work to be carried out next year.

It is recommended[2] that the study of methods for the determination of the pyrethrins in pyrethrum powder and for the analysis of derris and cube preparations be continued.

NOTE ON THE DETERMINATION OF FLUORINE

By J. J. T. GRAHAM (Insecticide Division, U. S. Food and Drug Administration, Washington, D. C.)

This Division, in the regular course of inspection work, had occasion to examine a sample that the manufacturer claimed contained 20 parts of barium fluosilicate and 80 parts of inert material. Analysis of the sample

[1] *Loc. cit.*
[2] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 48 (1937).

by the Willard-Winter method, *Methods of Analysis, A.O.A.C.*, 1935, 46, **19 (b)**, gave 5.37 per cent fluorine (equivalent to 13.16 per cent $BaSiF_6$), but the barium determination gave results equivalent to 21.64 per cent of barium fluosilicate.

Qualitative tests indicated that the inert material consisted essentially of kaolin, and it was believed that the fluorine determination was too low. In an attempt to avoid interference by the aluminum and silica in the kaolin the method was modified to include a preliminary distillation of 300 cc., at a temperature of $160° \pm 5°$. This distillate was made alkaline with sodium hydroxide and concentrated to a small volume, after which it was transferred to the distillation flask, and about 175 cc. was distilled at $135° \pm 3°$, as in the regular procedure.

The results by this modification, while higher than those previously obtained, were still too low to account for all the barium.

The lead chlorofluoride method[1] was then tried, after fusion with sodium-potassium carbonate and silica. The results by this method checked well with those obtained in the barium determination.

A synthetic sample, resembling the sample mentioned previously, was made by mixing 20 parts of barium fluosilicate of known purity with 80 parts of fullers' earth. This sample was passed through an 80-mesh sieve and thoroughly mixed, and analyses were made by the modified Willard-Winter method and by the lead chlorofluoride method.

The results are given in the table:

Fluorine results

SAMPLE	WILLARD-WINTER METHOD	WILLARD-WINTER METHOD (MOD.)	LEAD CHLOROFLUORIDE METHOD
	per cent	*per cent*	*per cent*
Commercial	5.37	7.51	9.04
		7.23	8.88
		Av. 7.37	9.00
			9.01
			Av. 8.98†
Synthetic		6.11	7.86
		6.17	7.78
		6.00	7.70
		6.00	—
		Av. 6.07*	7.78*

* Fluorine calculated from determination on $BaSiF_6$ = 7.79.
† Equivalent $BaSiF_6$, 22.00%; calculated from barium determination, 21.64%.

The results show that for samples containing a large percentage of aluminum and silica the Willard-Winter method gives low results. They also show that correct results can be obtained by the lead chlorofluoride method after fusion of the sample with alkali carbonates and silica.

[1] Bur. Standards Research Paper No. 110.

RECOMMENDATIONS[1]

It is recommended—

(1) That the Willard-Winter method for the determination of fluorine be modified by the addition of the warning, "Not applicable in the presence of aluminum silicate."

(2) That the lead chlorofluoride method for the determination of fluorine be studied next year.

REPORT ON FLUORINE COMPOUNDS

By R. H. CARTER (Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, Washington, D. C.), *Associate Referee*

The Willard-Winter[2] method as modified by Armstrong[3] is generally used in this laboratory for the examination of insecticides and spray residues containing fluorine. This method consists essentially of a separation of the fluorine from interfering materials by a distillation and a titration of an aliquot part of the distillate with thorium nitrate solution. Sodium alizarin sulfonate is used as indicator.

The adjustment of the *p*H of the solution to be titrated has been recognized as an important step in this procedure. In the Associate Referee's study of this point solutions containing known amounts of fluorine were buffered with Clark and Lubs[4] buffer solutions at *p*H values ranging from 1.0 to 3.8 and then titrated with standard thorium nitrate. It was found that at *p*H values from 1.6 to 3.0 the titration values decreased almost linearly with increase in *p*H. In other words, the titrations in solutions buffered at *p*H 3.0 were about 20 per cent lower than titrations of solutions buffered at *p*H 1.6. Below a *p*H value of 1.2–1.4, the titrations were considerably higher and above 3.0–3.4 there was a marked decrease.

Hoskins and Ferris[5] have recently investigated this phase of the titration, and they state that a *p*H of 3.5 is the most favorable when monochloroacetic acid is used as the buffer solution.

It is recommended[6] that the study of the optimum *p*H value for the titration with thorium nitrate be continued.

No formal report on disinfectants was given by the referee.

REPORT ON SUGARS AND SUGAR PRODUCTS

By C. A. BROWNE (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

Two years ago the Referee on Sugars and Sugar Products submitted some results of cooperative studies showing the effect of the two methods

[1] For report of Subcommittee A and action of the Association, see *This Journal* 20, 48 (1937).
[2] *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).
[3] *J. Am. Chem. Soc.*, 55, 1741 (1933).
[4] Olsen, J. C., Van Nostrand's Chemical Annual, p. 77 (1934).
[5] *Ind. Eng. Chem. Anal. Ed.*, 8, 6 (1936).
[6] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 48 (1937)

of clarification with basic lead acetate solution and dry basic lead acetate upon the polarization of various sirups and food products. The results of this work showed that while dry lead clarification reduced the volume of precipitate error caused by the use of basic lead solution, there was still a considerable increase in error in the direct polarization of these products, due to the action of the salts of lead partly by precipitating optically active constituents of the juice and partly by affecting their specific rotation. This is especially the case with fruit sirups, jellies, etc., which contain pectin, and it is because of the presence of this constituent that the procedures recommended for sugar cane and sugar beet products do not always apply to the widely diversified class of food products in which this Association is interested. In such cases a clarification with alumina cream, and, wherever necessary, a decolorization with a minimum quantity of activated vegetable carbon are preferable to a clarification with salts of lead or mercury.

With regard to the polarization of raw, cane and beet sugars and the correction of the polariscope readings for the excess error produced by the volume of precipitate in wet lead clarification, it was pointed out by the Referee at the 1919 meeting that this plus error was offset by the counterbalancing minus error of 0.1° in the graduation of the old Ventzke scale and that one of these errors, therefore, should not be corrected without at the same time correcting for the other. Under these conditions the claims of injustice by the advocates of scale correction or of lead precipitate correction alone were wholly unfounded in the polarization of the average class of raw sugars. At the present time, the minus 0.1 scale error upon many instruments has been corrected, and in such cases the positive counterbalancing error produced by wet lead clarification should also be corrected and this is easiest done by the method of dry lead clarification recommended by Horne, *Methods of Analysis, A.O.A.C.* 1935, 468, **18** (c).

The referee work of this Association upon sugars has been confined largely to determinations of the trisaccharide raffinose, the disaccharides sucrose, lactose and maltose, and to the common hexose monosaccharides dextrose and levulose. There are present, however, in food products, other important compounds, closely related to the sugars, for the determination of which methods have not yet been prescribed by this Association.

In January 1903, the present Referee[1] announced the discovery in cider vinegar of acetyl-methyl carbinol, a substance that reduces Fehling's solution in the cold and that can be regarded as the dimethyl derivative of the diose sugar glycolose. In April 1906, he[2] reported the presence of the same compound in a fermented sugar cane juice and expressed the belief that this compound was produced in many fermentations. This prediction has since been amply confirmed. Acetyl-methyl carbinol has been found not only in silage, buttermilk, bread, and many other food

[1] *J. Am. Chem. Soc.*, **25**, 31 (1903).
[2] *Ibid.*, **28**, 468 (1906).

products in which fermentation has occurred but in materials where carbohydrates have undergone destructive alteration, as in the pyroligneous distillate of woods. Balcom,[1] in 1916, gave a résumé of the work on the occurrence of this compound in cider vinegars. Later work on improved methods for its detection and estimation justifies the study of methods for its determination by this Association.

The method first followed by the Referee over 30 years ago consisted in precipitating and weighing acetyl-methyl carbinol as the highly insoluble phenyl osazone $C_{16}H_{18}N_4$, the weight of which multiplied by the factor 0.33 gives the approximate weight of carbinol. In another method devised by Arbenz[2] the distillate of the food product is mixed with Fehling's solution and allowed to stand in the cold overnight. The precipitated cuprous oxide is filtered off, and its weight multiplied by the factor 0.25 gives the approximate amount of acetyl-methyl carbinol.

A more delicate method for determining acetyl-methyl carbinol is that of Lemoigne,[3] which is based upon its oxidation with ferric chloride to diacetyl, which after distillation is converted by means of hydroxylamine into diacetyl-dioxime. The latter compound is then precipitated with nickel chloride as nickel diacetyl-dioxime, the weight of which multiplied by 0.61 gives the weight of acetyl-methyl carbinol. The reactions involved in these conversions are as follows:

$$\begin{array}{c} CH_3 \\ | \\ HCOH \\ | \\ C=O \\ | \\ CH_3 \end{array} + 2FeCl_3 = \begin{array}{c} CH_3 \\ | \\ C=O \\ | \\ C=O \\ | \\ CH_3 \end{array} + 2FeCl_2 + 2HCl.$$

Acetyl-methyl carbinol Diacetyl

$$\begin{array}{c} CH_3 \\ | \\ C=O \\ | \\ C=O \\ | \\ CH_3 \end{array} + \quad 2H_2NOH \quad = \begin{array}{c} CH_3 \\ | \\ C=NOH \\ | \\ C=NOH \\ | \\ CH_3 \end{array} + 2H_2O$$

Diacetyl + Hydroxylamine = Diacetyl-dioxime.

$$2 \begin{array}{c} CH_3 \\ | \\ C=NOH \\ | \\ C=NOH \\ | \\ CH_3 \end{array} + \quad NiCl_2 \quad = \begin{array}{ccccc} CH_3 & & & & CH_3 \\ | & & & & | \\ C=NOH & & & HON= & C \\ | & & & & | \\ C=NO & — & Ni & —ON= & C \\ | & & & & | \\ CH_3 & & & & CH_3 \end{array} + 2HCl$$

Diacetyl-dioxime + Nickel chloride = Nickel dimethyl-dioxime.

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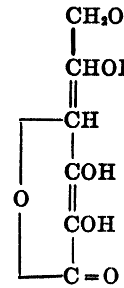
[1] *J. Am. Chem. Soc.*, 39, 309 (1917).
[2] *Mitt. Lebensm.*, 15, 52 (1924).
[3] *Compt. rend.*, 170, 131–2 (1920).

Inasmuch as diacetyl itself frequently accompanies acetyl-methyl carbinol in food products a blank distillation should always be conducted without the use of ferric chloride as an oxidizing agent. The difference between the weights of the nickel dioxime obtained in the two distillations gives the amount due to the presence of acetyl-methyl carbinol.

In addition to acetyl-methyl carbinol there is another important reducing substance, closely related to the sugars, which occurs in a wide range of food products. This is ascorbic acid, or vitamin C, concerning the constitution and synthesis of which so much has appeared in the chemical literature of recent years. The formula for ascorbic acid is $C_6H_8O_6$, its atomic structure being indicated by the following arrangement:



Ascorbic acid in food products is usually determined by the method of Tillmans, which depends upon the titration of its reducing power in the cold with 2.6-dichlorophenol indophenol. The determination of ascorbic acid in food products belongs to the subject of vitamins and is not considered further in this report.

Another sugar derivative of high reducing power that plays an important role in the chemistry of certain food products is glucic acid, a very unstable compound that is produced by the action of alkalies upon glucose. Glucic acid was first prepared by Winter in Java in 1894 by warming an aqueous solution of 1 per cent invert sugar in the absence of the air with 0.5 per cent of calcium oxide (which had first been slaked). The calcium glucate thus precipitated was filtered off, decomposed with dilute sulfuric acid, and extracted with ether. On evaporating the ether solution, the glucic acid was obtained in crystalline form. Winter did not determine the formula of this new compound. He called attention to its high reducing power (Fehling's solution being reduced in the cold), to its acidic properties, and to its great instability, formic acid being reported among the products of decomposition.

In an investigation of the decomposition products of sugar cane molasses in 1928 the Referee had occasion to repeat this work of Winter. The problem of isolating and identifying Winter's glucic acid was entrusted to E. K. Nelson of the Bureau of Chemistry and Soils, who found the compound to have the formula $C_3H_4O_3$ (one-half that of ascorbic

acid), for which, because of its highly acidic and reducing properties, the following structure was suggested:

$$\begin{array}{l} \mathrm{CHOH} \\ \| \\ \mathrm{CH} \\ | \\ \mathrm{COOH} \end{array}$$

This is the enolic form of the semi-aldehyde of malonic acid. It was surmised at the time that this compound, because of its high reduction potential and acidic properties, might be related to ascorbic acid. Feeding tests upon guinea pigs, conducted by Nelson and Mottern[1] of the Bureau of Chemistry and Soils, showed, however, that glucic acid had no antiscorbutic properties.

In 1933, Euler and Martius,[2] by the action of sodium hydroxide upon glucose in the presence of lead acetate and the decomposition of the precipitated lead salt with hydrogen sulfide, prepared a compound that was similar in composition ($C_3H_4O_3$) and in its acidic, reducing, and non-antiscorbutic properties to the glucic acid investigated by Nelson and Browne. Euler and Martius ascribed to their compound, which they called "reducton" or oxy-malonic dialdehyde, the structural formula,

$$\begin{array}{r} \mathrm{H{-}COH} \\ \| \\ \mathrm{COH} \\ | \\ \mathrm{HC{=}O}, \end{array}$$

the acidity being ascribed to the activated enolic group and not to a carboxyl group, as had been supposed by Nelson and Browne. Subsequent investigations by Nelson show that the compound first isolated by Winter in 1894 and that prepared by Euler and Martius in 1933 are identical. On grounds of priority, therefore, it would seem that the name glucic acid employed by Winter and retained by Nelson and Browne should keep its place in the literature. The alteration of a constitutional formula does not justify the obliteration of previous non-descriptive names. Ascorbic acid, with all the changes that have been suggested in its structural formula, is still called ascorbic acid, although constitutionally it is not an acid.

Glucic acid, although formed in the alkaline clarification of sugar-cane juices, is speedily broken down into formic acid and other decomposition products that are found in the by-products of sugar manufacture, such as sirup and molasses. As pointed out by Nelson and Browne, it absorbs atmospheric oxygen so vigorously that considerable heat is evolved. The presence of glucic acid may, therefore, be an explanation of the so-called

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[1] *Ind. Eng. Chem.*, 25, 217 (1933).
[2] Ann., 505, 73 (1933); also *Arkiv Kemi, Mineral. Geol.*, 11B, No. 14.

hot room or froth-fermentation of molasses. The formation of lead glucate and its rapid oxidation may possibly explain the reported occurrences of the spontaneous ignition of the precipitates obtained by clarifying sugar solutions with basic lead solutions.

The action of lime on the glucose and fructose of sugar cane and other plant juices results in the production of other reducing substances besides glucic acid. Among these may be mentioned the so-called unfermentable sugars of molasses, which have given rise to so many disputes between buyers and sellers of this product. The distiller, who buys molasses on the basis of its sugar content, naturally objects to paying for sugar that does not ferment. The name glutose was applied by Lobry de Bruyn and van Ekenstein[1] to the unfermentable sugar obtained by the treatment of glucose, fructose, and mannose with dilute alkalies or with hydrated oxide of lead. From 1 to 5 per cent of glutose is said by Pellet[2] to occur in sugar cane molasses. Glutose is reported to have only about half the reducing power of glucose, but considerable doubt exists as to the accuracy of the methods for its estimation. The presence of reducing sugars in the slop from fermented molasses is not an absolutely true index of unfermentable sugar. In the Referee's experience such slops, when clarified and subjected again to the action of yeast, may undergo a second fermentation with further loss of sugar. Inhibiting substances, removed by clarification, may perhaps have interfered with the completion of the primary fermentation.

RECOMMENDATIONS

In planning the work for next year the Referee on Sugars and Sugar Products would recommend[3] (1) a study of methods for determining acetyl-methyl carbinol and diacetyl in food products and (2) a study of methods for determining the so-called unfermentable sugars of molasses.

No report on honey was given by the associate referee.

REPORT ON MAPLE PRODUCTS*

By J. F. Snell (Macdonald College, Province of Quebec, Canada), *Associate Referee*

In accordance with the recommendations adopted last year, *This Journal*, **19**, 48, 399 (1936), study of concentrates of maple flavors, true and imitation, and collaborative work on the Jones device for amelioration of filtration in the determination of Canadian lead values were continued.

[1] *Rec. trav chim.*, **16**, 62, 282 (1897).
[2] *Bull assoc. chim sucr. dist.*, **16**, 1181 (1899); **19**, 834 (1902).
[3] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 62 (1937)
* Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Quebec, Canada. Journal Series No. 80.

The Chemistry Division of the National Research Council (Canada) collected samples of six preparations of imitation flavors in addition to the nine referred to in last year's report. Comparisons of these with the concentrates made from genuine maple sirups were made in the laboratory of the Macdonald College Chemistry Department. This work is not sufficiently advanced to warrant description at this time.

The device of adding dry asbestos to the precipitation vessel before filtering the Canadian lead precipitate, *This Journal*, **19**, 398 (1936), was tried again in the laboratory of the Cary Maple Sugar Company, St. Johnsbury, Vermont, and also in three other laboratories. In three samples giving lead values of 3.15, 4.13, and 4.87, Augustus Conlin of the Cary Company found the average time required for filtration to be reduced, respectively, from 29 to 10, 32 to 13, and 43 to 17 minutes when the asbestos was used. In the first and third samples the agreement between duplicates was also improved. In the Chemistry Division of the Dominion Experimental Farms, Ottawa, G. B. Moses with sirups of lead values 4.71, 4.82, and 5.84 found that while with the official method 14–15 minutes was required for filtration, only 9–10 minutes was required when asbestos was added. In two of the three samples agreement between duplicates was better with than without the asbestos. E. H. Berry of the U. S. Food and Drug Administration, Chicago, also found in three samples that the addition of asbestos slightly lessened the time required for filtration, but thought this advantage was offset by the additional time required for preparation of the asbestos. The agreement of duplicates was not materially improved, except in a sample showing the low lead value of 0.87 (and doubtless, therefore, not maple sirup). Roger Gale of the General Foods Corporation Eastern Development Laboratory, Hoboken, N. J., found that in sirups with lead values of 3.95, 2.65, and 2.70, the average time required for filtration was reduced, respectively, from 4.3 to 3.1, 5.8 to 2.9, and 4.0 to 3.4 minutes. Duplicates by both methods agreed. In a few instances collaborators found it necessary to extend the time of drying of the precipitate on account of the added asbestos. In no instance did the lead values obtained with and without the use of the asbestos differ materially.

An alternative suggestion of Jones that the asbestos might be added after the supernatant liquid is decanted from the precipitate was tried in the four laboratories mentioned previously and in Macdonald College, but it was found less advantageous.

As all collaborators, in six laboratories, including that of Jones, found filtration and washing facilitated by the use of the asbestos—though one of them thinks this advantage offset by the time required for the preparation of the asbestos—and as it appears to obviate the danger of formation of fissures in the precipitate in the Gooch, it is recommended—[1]

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 62 (1937).

(1) That the following note be added to the directions for determination of Canadian lead value, *Methods of Analysis, A.O.A.C.*, 1935, **XXXIV**, 114, 492:

NOTE: Filtration may be facilitated and the necessity of keeping the precipitate covered with water in the crucible obviated by stirring a weighed quantity (0.5 gram or less) of dry asbestos fiber with the precipitate and supernatant liquid shortly before filtration.

(2) That the study of maple flavor concentrates and imitations be continued.

No report on drying, densimetric, and refractometric methods was given by the associate referee.

No separate report on polariscopic methods was given by the associate referee. See report of the referee.

No report on chemical methods for reducing sugars was given by the associate referee.

REPORT ON LEAD PRECIPITATE

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*

Owing to lack of available time it was not possible to carry out the full program recommended in last year's report, *This Journal*, **19**, 401, (1936).

Only twelve raw sugars were subjected to the suggested tests, by C. A. Gamble. Two of the samples were from Cuba, three from Puerto Rico, four from the Philippines, and one each from Hawaii, Florida, and St. Croix. As in similar experiments reported previously, *This Journal*, **18**, 178 (1935), a double normal stock solution of each sugar was prepared and decolorized with a minimum quantity of activated carbon. The filtrate was diluted to normal concentration. One portion was read directly in the saccharimeter. Other portions of 100 ml. each were first clarified with mixtures of Horne's dry lead and dry mercuric acetate, and the filtrates were polarized. The following average results were obtained:

Clarifying agents used	*Average polarization*
gram *gram*	*°V.*
None	97.55
0.2 dry lead, 0.1 dry mercuric acetate	97.64
0.2 dry lead, 0.2 dry mercuric acetate	97.60
0.2 dry lead, 0.3 dry mercuric acetate	97.59

The mixture of 0.2 gram of dry lead and 0.1 gram of dry mercuric acetate raised the average actual polarization by 0.09°V. Larger amounts of

dry mercuric acetate progressively diminished the increase in the polarization caused by the lead subacetate, but the true polarization was not reached even with 0.3 gram of mercuric acetate. The latter quantity gave practically the same result as did 0.2 gram, and therefore in order to duplicate the true polarization it would appear to be necessary to reduce the amount of Horne's dry lead rather than to increase that of dry mercuric acetate.

It is recommended[1] that this investigation be continued along the lines indicated, with a large enough number of samples to permit definite conclusions to be drawn.

No report on vinegars was given by the referee. The recommendations presented are given in *This Journal*, **20**, 61 (1937).

REPORT ON ASH IN VINEGAR

By HARRY SHUMAN (U. S. Food and Drug Administration, Philadelphia, Pa.), *Associate Referee*

J. O. Clarke, *This Journal*, **9**, 440 (1926), first pointed out through collaborative study that the results for total and soluble ash and for soluble and insoluble P_2O_5 varied widely; that insoluble ash and alkalinity gave concordant results; and that total P_2O_5 was more uniform than soluble and insoluble P_2O_5. He suggested the possible desirability of replacing soluble and insoluble P_2O_5 by total P_2O_5. Subsequent studies by Clarke, and by Clarke and Feldbaum, *This Journal*, **10**, 490 (1927); **11**, 499 (1928), showed that "temperature of ashing within reasonable limits was not of great importance" for total ash, but that large increases in temperature resulted in considerable losses. It was also shown that small variations in ashing temperature changed the ratio between soluble and insoluble P_2O_5, although the total P_2O_5 was practically unaffected. Henry, *This Journal*, **13**, 471 (1930); **16**, 536 (1933), studied the effect of ashing temperatures of 500°, 550°, and 600° C., using a muffle with automatic temperature control. He found that temperature may be disregarded between the range of 500°–600° C. for ash and subsequent ash determinations, except soluble and insoluble P_2O_5. In general, increase in temperature increased soluble P_2O_5 and decreased insoluble P_2O_5. Studies on the use of sucrose to reduce the time of ashing gave indications in its favor.

In his study on ash the Associate Referee included both cider and malt vinegars, because the ash of these vinegars differs materially in several respects. The alkalinity of the ash of malt vinegar is much lower than that of cider vinegar, and the P_2O_5 is much higher.

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 62 (1937).

Ashing was done in a muffle equipped with an automatic temperature control. The floor of the furnace was covered with a sheet of quarter-inch asbestos because uneven redness indicated lack of uniformity in the temperature. Otherwise superheating of some determinations might occur when parts of the muffle were of higher temperature than that recorded by the instrument, the thermocouple of which is located in the center of the furnace chamber and is heated by the surrounding air. The temperatures used may be described as from faint redness (500°), through dull redness (550°), to low redness (600° C.).

Ashing method **58 (b)**, *Methods of Analysis, A.O.A.C.*, 1935, 456, was used because this modification lends itself to possible shortening by the use of sucrose. Parallel determinations were made with and without sucrose. The sugar (ash-free) was dissolved in the vinegar, evaporated on the steam bath, and charred over a Bunsen burner, and ashing was continued as directed in method **58 (b)**. Two grams of sucrose was used because this amount produces a light porous char.

SUCROSE

Results can not be regarded as particularly encouraging to the use of sucrose as is seen by a comparison in Tables 1 and 2 of a number of moistening treatments. Usually the number of moistenings required to produce a carbon-free ash are not substantially reduced by its addition; with cider vinegars, the number of moistenings are generally decreased by one, at 500° and 550° C.; and at 600° C. an equal number of moistenings are required with and without sucrose. Although most of the char is rapidly burned off when sucrose is used, there usually remains a small amount of resistant carbonaceous material that requires additional treatment, so that finally there is little gained by its use. With malt vinegars there is very little gained. In some cases, sucrose slows up ashing. Sucrose does not materially affect the results of ash determinations as obtained without its use, except in the case of soluble P_2O_5 of malt vinegars, which is increased by its presence.

TEMPERATURE

The effect of temperature within the range of 500°–600° C. may be generally disregarded. If those determinations in which temperature alone is a factor (Table 1) are considered, the values for total, soluble and insoluble ash and for alkalinity are identical in most cases at 500° and 550° C. for cider vinegars. The fact that values for total ash are only slightly lower at 600° C. for cider vinegars indicates that 600° C. is probably the highest safe temperature.

Determinations for malt vinegar, except for total ash, were made at 550° and 600° C. only. Values for total ash are slightly higher at 500° C. than at 550° and 600° C. for vinegars No. 1 and 2. However, with vinegar

TABLE 1.—*Effect of temperature on the composition of ash in vinegar*

TEMPERATURE OF ASHING	NUMBER OF MOISTENINGS	TOTAL ASH	SOLUBLE ASH	INSOLUBLE ASH	ALKALINITY OF SOLUBLE ASH	SOLUBLE P_2O_5	INSOLUBLE P_2O_5	TOTAL P_2O_5 (SOLUBLE +INSOLUBLE)	SOLUBLE P_2O_5 AS % OF TOTAL
°C.		g./100 cc.	g./100 cc.	g./100 cc.	cc.N NaOH/100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	
Cider Vinegar No. 1									
500	5	0.30	0.27	0.026	3.4	12.0	11.4	23.4	51
550	4	0.30	0.27	0.027	3.4	13.0	9.5	22.5	58
600	3	0.29	0.26	0.028	3.4	14.4	8.0	22.4	64
Cider Vinegar No. 2									
500	4	0.32	0.28	0.036	3.6	4.0	11.0	15.0	27
550	2	0.32	0.28	0.036	3.7	6.8	8.7	15.5	44
600	1	0.30	0.27	0.036	3.6	8.5	7.0	15.5	55
Cider Vinegar No. 3									
500	4	0.33	0.30	0.032	4.0	4.3	11.2	15.5	28
550	3	0.33	0.30	0.034	4.0	5.7	9.0	14.7	39
600	1	0.32	0.29	0.031	4.0	7.8	7.0	14.8	53
Malt Vinegar No. 1									
500	1	0.34	—	—	—	—	—	—	—
550	0	0.33	0.18	0.15	0.6	17.7	54.3	72.0	25
600	0	0.33	0.19	0.14	0.8	25.1	46.5	71.6	35
Malt Vinegar No. 2									
500	4	0.29	—	—	—	—	—	—	—
550	2	0.28	0.089	0.19	0.4	5.9	78.8	84.7	7
600	1	0.28	0.096	0.18	0.4	5.1	72.5	77.6	7
Malt Vinegar No. 3									
500	2	0.32	—	—	—	—	—	—	—
550	2	0.30	0.13	0.17	0.4	9.4	66.3	75.7	12
600	0	0.29	0.12	0.17	0.4	8.7	66.2	74.9	12

No. 3 there is a difference of 0.03 gram per 100 cc. between 500° and 600° C. The results for soluble and insoluble ash and alkalinity are in good agreement.

Only one vinegar (Table 1) shows a difference for total ash greater than 0.01 gram per 100 cc. between temperatures of 500° and 550° C. This same vinegar is also the only one that shows a difference of more than 0.02 gram per 100 cc. between temperatures of 500° and 600° C.

Ashing at 500° C. is generally slow and tedious, and seldom is the ash entirely free from all traces of carbon even with repeated moistenings. At 550° C. ashing is far more satisfactory, and there is little need for "ash aids."

TABLE 2.—*Sucrose as an ash aid and its effect on composition of ash*
(Results to be compared with Table 1)

TEMPERATURE OF ASHING	NUMBER OF MOISTENINGS	TOTAL ASH	SOLUBLE ASH	INSOLUBLE ASH	ALKALINITY OF SOLUBLE ASH	SOLUBLE P_2O_5	INSOLUBLE P_2O_5	TOTAL P_2O_5 (SOLUBLE + INSOLUBLE)	SOLUBLE P_2O_5 AS % OF TOTAL
°C.		*g./100 cc.*	*g./100 cc.*	*g./100 cc.*	*cc. N NaOH/100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	
				Cider Vinegar No. 1					
500	4	0.30	0.27	0.027	3.4	12.4	10.9	23.3	53
550	3	0.30	0.27	0.034	3.3	12.9	9.5	22.4	58
600	3	0.29	0.26	0.032	3.3	13.6	9.1	22.7	60
				Cider Vinegar No. 2					
500	3	0.31	0.27	0.038	3.5	4.9	10.2	15.1	32
550	2	0.31	0.27	0.038	3.6	6.8	8.4	15.2	45
600	1	0.30	0.26	0.035	3.5	8.0	7.4	15.4	52
				Cider Vinegar No. 3					
500	3	0.31	0.28	0.033	3.8	3.9	10.9	14.8	26
550	2	0.33	0.29	0.035	4.0	5 0	9.8	14.8	34
600	1	0.32	0.29	0.033	4.0	6.4	8.7	15.1	42
				Malt Vinegar No. 1					
550	1	0.33	0.18	0.15	0.8	24.6	49.1	73.7	33
600	0	0.33	0.18	0.15	0.8	24.9	47.1	72.0	35
				Malt Vinegar No. 2					
550	2	0.27	0.084	0.19	0.5	9.9	77.1	87.0	11
600	1	0.27	0.081	0.19	0.5	9.9	76.8	86.7	11
				Malt Vinegar No. 3					
550	1	0.30	0.14	0.16	0.5	12.0	62.2	74.2	16
600	1	0.29	0.13	0.16	0.5	14.4	69.4	83.8	17

DESICCATION AND WEIGHING OF ASH

The handling of the ash, following incineration, namely, desiccation and weighing, is of vital importance, as may be seen from Table 3. These results were obtained on a humid day in August and perhaps represent extreme conditions, but nevertheless they are indicative of what may occur to a lesser extent throughout the year. It is significant that these difficulties occur with cider vinegars, but not with malt vinegars. The high alkalinity of cider vinegar, due chiefly to potassium carbonate, which in its anhydrous state is very hygroscopic, is doubtless the cause. On the other hand, the relatively low alkalinity of malt vinegar is in keeping with its non-hygroscopic nature.

The procedure followed in this work is that indicated in Table 3. Desiccators containing "new" sulfuric acid were used, and only two dishes were cooled in any one desiccator. The dishes were rapidly weighed, then

TABLE 3.—*Possible effect on total ash of differences in desiccation*

DESICCATION*	ORDER OF WEIGHING	CIDER VINEGAR A		CIDER VINEGAR B	
		1	2	3	4
Cooled in single desiccator containing "old" H_2SO_4	1, 2, 3, 4	.38	.35	.37	.43
Cooled in single desiccator containing "new" H_2SO_4	1, 2, 3, 4	.34	.35	.38	.37
1, 2, in desiccator containing "new" H_2SO_4 3, 4, in desiccator containing "old" H_2SO_4	1, 2, 3, 4	.33	.33	.35	.37
1, 2, and 3, 4, in separate desiccators containing "new" H_2SO_4	1, 2, 3, 4	.33	.34	.34	.34

* Dishes reheated in muffle for 5 minutes between desiccations.

replaced in the muffle for several minutes, and cooled again in the desiccator. With weight previously obtained on the balance pan each dish was again rapidly weighed to nearest milligram.

Other procedures, perhaps more reliable, have suggested themselves. A dish with a tightly fitting cover might be ideal, but few platinum dishes are thus equipped. Several experiments were made with aluminum foil and tin foil, such as the so-called bottle caps used in milk analysis. The foil was shaped to fit closely over the platinum dish and kept in the desiccator until ready for use. The platinum dish, after removal from the muffle, was placed on an asbestos pad, and as soon as it could be safely touched was quickly covered with the shaped tin foil cover and placed in the desiccator. The covered dish and ash were weighed, and finally the weight of the dish and cover was subtracted from the total weight. An ash determined in the usual way (one weighing) weighed 0.0880 gram. When the foil cover was used, the weight obtained was 0.0835, a difference of 4.5 mg., sufficient to change the ash from 0.35 to 0.33 gram per 100 cc.

SOLUBLE AND INSOLUBLE P_2O_5 VS. TOTAL P_2O_5

The results (Table 1) obtained for soluble and insoluble P_2O_5 in cider vinegars confirm previous findings that these determinations are sensitive to changes in ashing temperatures. Increase in ashing temperature is accompanied by an increase in soluble P_2O_5 and a corresponding decrease in insoluble P_2O_5. The total P_2O_5 appears to be little affected.

Malt vinegars are somewhat different. No determinations were made at 500° C., but the results at 550° and 600° C. are conclusive. Only malt

vinegar No. 1 shows a marked change of soluble and insoluble P_2O_5 with ashing temperatures. The low result obtained for insoluble P_2O_5 with malt vinegar No. 2 suggested possible loss of P_2O_5 in ashing, and the need for employing fixatives. Accordingly, total P_2O_5 was determined as follows: 25 cc. of vinegar was evaporated with 2 cc. of 50 per cent magnesium nitrate solution (1 cc. for cider vinegars as with 2 cc. the combustion is violent). The ash was dissolved in 10 cc. of nitric acid (1+1), made to 100 cc. and filtered, and P_2O_5 was determined on an aliquot part. Several determinations were also made by digestion with nitric and sulfuric acids. A comparison of the results obtained, with total P_2O_5 by ordinary ashing, is shown in Table 4.

TABLE 4.—*Total P_2O_5 (mg. per 100 cc.)*

VINEGAR	ORDINARY ASHING AT 550° C	ASHING WITH $Mg(NO_3)_2$ AT 550° C.	DIGESTION WITH HNO_3 AND H_2SO_4
Cider No. 1	22.5	23.0	—
Cider No. 2	15.5	16.0	15.4
Malt No. 1	72.0	73.8	73.4
Malt No. 2	84.7	109.3	109.3
Malt No. 3	75.7	97.0	—

While the loss of P_2O_5 in ordinary ashing appears to be slight for cider vinegars, it is considerable for malt vinegars, and further emphasizes the unreliability of soluble and insoluble P_2O_5 determinations.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the use of sucrose or other substances for reducing the time of ashing be dropped.

(2) That determination of total ash be studied collaboratively, with particular attention given to desiccation, weighing, and the use of suitable covers for the ashing dish.

(3) That the determination of total phosphoric acid in the presence of suitable fixatives be studied collaboratively, and that further studies on soluble and insoluble P_2O_5 be discontinued because of the accumulated evidence of their unreliability.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

In order to test the applicability of the present methods for vanilla when applied to an imitation vanilla flavor having glycerol as its solvent,

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 61 (1937).

the Referee prepared an imitation vanilla flavor having the following composition:

	Formula	*Composition*
Vanillin	311 g.	0.622 g. per 100 cc.
Coumarin	60 g.	0.120 g. per 100 cc.
Vanilla	30 cc.	6 cc. per 100 cc.
Glycerol	220 g.	35.6 cc. per 100 cc.
Caramel	to color	
Water	q. s.	
	500 cc.	

The proportion of glycerol in 100 cc. of flavor was estimated in the following manner: The glycerol used had a sp. gr. of 1.2518 at 25°/25° C., corresponding to 96.06 per cent glycerol according to the table given on page 158 of the book, "Glycerol and the Glycols," by James W. Lawrie. In the U. S. P. the lowest limits permitted are sp. gr. 1.2491 at 25°/25° C. and glycerol content of 95 per cent. Therefore, 1 cc. of U. S. P. glycerol contains not less than 1.186 grams of pure glycerol and 220 g. $\times$ 0.9606 $\div$ 1.186 = 178.2 cc. of U. S. P. glycerol in a total volume of 500 cc., or 35.6 cc. U. S. P. glycerol per 100 cc. of flavor.

Two collaborators examined the sample by the following procedure:

EXAMINATION OF IMITATION VANILLA IN A VEHICLE OF GLYCEROL

Alcohol

Measure at room temperature 100 cc. of sample into a suitable distilling flask, add 60 cc. of water, and distil 90–95 cc. into a volumetric flask. Fill the receiving flask to the mark and determine alcohol by means of the specific gravity at a convenient temperature.

Transfer the residue from the distillation flask into a 100 cc. volumetric flask and make up to the mark with water at room temperature (Soln. A).

Glycerol

Determine the apparent specific gravity of Solution A at 15°/15°, 20°/20°, or 25°/25° and find the per cent glycerol from Table 21 ("Glycerol and the Glycols," by James W. Lawrie, page 158). To find cc. U. S. P. glycerol per 100 cc. of sample, multiply the specific gravity of Solution A by the per cent glycerol and divide by 1.186.

Vanillin and Coumarin

Pipet 50 cc. of Solution A into a 100 cc. volumetric flask, add 25 cc. of 8% lead acetate soln., make up to the mark, mix, and hold overnight at a temperature of 37°–40° C. Filter, and determine vanillin and coumarin as directed in the official method, *Methods of Analysis, A.O.A.C.*, 1935, 306.

The results obtained by the collaborators are given in Table 1.

TABLE 1.—*Results of analysis of imitation vanilla flavor in a vehicle of glycerol*

	JOHN B. WILSON	R. A. OSBORN	PRESENT
Alcohol, by volume (%)	2.54	2.80	2.70
Glycerol per 100 cc. (cc.)	37.57	37.63	35.6
Vanillin per 100 cc. (g.)	0.60	0.62	0.62
Coumarin per 100 cc. (g.)	0.11	0.10	0.12

The Referee also determined vanillin and coumarin without adding lead acetate. Considerable difficulty was experienced with emulsions, but the results check those obtained above, namely, vanillin 0.60 g., coumarin 0.10 g. per 100 cc.

These data show (1) that vanillin and coumarin can be recovered from imitation vanilla in a menstruum of glycerol with a fair degree of accuracy by the official gravimetric method, and (2) that the specific gravity method shows promise as a rapid method for determination of glycerol in imitation vanilla.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative work be done on the determination of vanillin and coumarin in imitation vanillas.

(2) That a further study be made of the possibility of determining glycerol in vanilla flavors by the specific gravity method.

REPORT ON MEAT AND MEAT PRODUCTS

By R. H. KERR (U. S. Bureau of Animal Industry, Washington, D. C.), *Referee*

Further studies of methods for the determination of nitrates, soybean flour, dried skim milk, and benzoates in meat and meat food products were made. No samples were distributed to collaborators.

Recommendation is renewed for adoption as tentative of the two methods for the qualitative detection of soybean flour in sausage and other meat products presented at the last meeting, *This Journal*, **19**, 409 (1936). These methods have been thoroughly tested in the Meat Inspection Laboratories of the Bureau of Animal Industry and found to be reliable within the limitations plainly indicated in them. They are being regularly used and relied upon for the qualitative detection of soybean flour.

In the course of an investigation conducted by W. C. McVey of the Bureau of Animal Industry, which involved the determination of benzoate of soda in sausage, need for a more sensitive and accurate method for quantitative determination of that substance than the one published in the *Methods of Analysis* arose. McVey successfully adapted a method published by Illing,[2] to the problem in hand. As this method applies generally to other foods as well as meat, its study is thought to be a matter for the attention of the Referee on Food Preservatives rather than the Referee on Meat and Meat Products.

No recommendations with respect to methods for nitrates and dried skim milk are presented at this time. It is intended to give these methods further study.

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, **20**, 65 (1937).
[2] *Analyst*, **57**, 224 (1932).

REPORT ON GINGER

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York), *Referee*

Work was continued in accordance with the recommendations approved last year. O. C. Kenworthy and B. Lubell of this Station collaborated.

Samples of coarsely ground African ginger and Jamaica ginger were given to the collaborators, together with last year's report and a method for the assay. The method was published in *This Journal*, **19**, 98 (1936).

Results of assay

	CLEVENGER		KENWORTHY*		LUBELL	
	1	2	1	2	1	2
Resins (%)	6.42	3.35	6.74	3.73	6.39	3.46
Volatile Oil (cc./100 g.)	3.25	1.39	2.46	1.22	3.30	1.37
Specific gravity (25°/25°)	0.879	0.884	0.888	0.884	0.879	0.884
Optical Rotation†	−42.2	−32.2	−21.8	−16.6	−41.1	−35.5
Refractive Index (20° C.)	1.491	1.493	1.494	1.495	1.492	1.494
Acid Number	2.93	4.29	2.6	4.8	3.5	3.46
Ester Number	11.02	18.7	35.3	25.3	16.03	17.7

* Analyses made 10 months after ginger was ground and stored in sealed glass jars.
† Angular degrees 25° C., 100 mm. tube, white light.

The results reported for ginger are considered satisfactory. Variations in the results reported are probably accounted for by the variation in the elapsed time between grinding of the ginger and the assay of the material.

It is recommended that the work on ginger be discontinued for the present.

No report on baking powders and baking chemicals was given by the referee.

No report on nuts and nut products was given by the referee.

REPORT ON FISH AND OTHER MARINE PRODUCTS

By H. D. GRIGSBY (U. S. Food and Drug Administration, Philadelphia, Pa.), *Referee*

No methods for the analysis of fish and other marine products have been adopted by this Association, but it is hoped that when the next edition of *Methods of Analysis* is published a reasonably complete set of methods for these products will be included in the chapter planned for them (Chapter XXIV).

This year the Referee is making a start by presenting methods for the examination of oysters and scallops. These methods, for preparation of sample, total solids, and total chlorides, are in practically the same form as those compiled by C. W. Harrison, A. M. Henry, and other members of the Food and Drug Administration. They were adopted, and are published in *This Journal*, **20**, 70 (1937).

It is generally considered necessary in the open Carius method for the determination of chlorine to remove the nitrous acid by addition of urea or hydrazin sulfate to avoid the formation of colored compounds with the thiocyanic acid, but the Referee's experience is that their use makes no material difference in the results. Five cc. of a saturated hydrazin sulfate solution may be added just before the addition of the ferric indicator.

The methods for oysters provide for a separate examination of free oyster liquor when the free liquor exceeds 10 per cent of the total volume. This excessive amount of liquor will only be found in packages of oysters that have been frozen after packing or to which water has been added.

The volume of the free liquor is determined by draining the meats on an oyster skimmer for 1 minute and measuring the shrinkage in the volume of the meats after they are returned to the original measuring vessel. The skimmer is a flat-bottomed metal tray with the bottom perforated with holes $\frac{1}{4}''$ in diameter spaced $1\frac{1}{4}''$ in a square pattern. The skimmer should be of sufficient size to take the volume of oysters drained at one time in a single layer.

This year at the Referee's request the methods presented were examined collaboratively in the Boston, New York, Baltimore, and Philadelphia laboratories of the U. S. Food and Drug Administration.

Instead of attempting to send out duplicate samples of scallops and oysters, and Referee requested each of the above laboratories to secure its own samples and then arrange for at least three analysts in each laboratory to analyze the same sample at the same time by the proposed methods.

The tabulated results cover eight samples, four of oysters and four of scallops, each sample analyzed by at least three analysts. The tabulations are separated under the headings of each of the four examining laboratories to show the agreement of the analysts in each case on the same sample.

It will be noted that the agreement in results on the same samples is reasonably close on all the methods tested and that the results for the ashing method for salt agree well with those of the open Carius method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods presented for examination of oysters and scallops, namely, Preparation of Sample, Total Solids, Sodium Chloride by

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 60 (1937).

Collaborative results

ANALYST	TOTAL SOLIDS	SODIUM CHLORIDE— BY OPEN CARIUS	SODIUM CHLORIDE— BY ASHING WITH CALCIUM ACETATE
Philadelphia Laboratory—Oysters			
	per cent	*per cent*	*per cent*
F. K. Killingsworth	16.21	0.48	0.47
	16.31	0.47	0.46
H. Shuman	16.20	0.48	0.46
	16.22	0.47	0.46
H. D. Grigsby	16.22	0.48	0.46
	16.35	0.47	0.46
Scallops			
D. M. Taylor	20.07	0.39	0.40
	19.97	0.39	0.40
J. J. Chap	20.15	0.40	0.40
	20.15	0.39	0.40
H. D. Grigsby	19.94	0.405	0.406
	19.94	0.405	0.406
New York Laboratory—Oysters			
J. L. Hogan	14.80	0.29	0.27
	14.65	0.29	0.27
D. B. Scott	14.72	0.30	0.28
	14.71	0.30	0.28
W. E. Kirby	14.68	0.30	0.26
	14.68	0.29	0.27
Scallops			
J. L. Hogan	21.22	0.53	0.51
	21.36	0.53	0.51
D. B. Scott	21.23	0.50	0.52
	21.42	0.50	0.52
W. E. Kirby	21.11	0.50	0.52
	21.11	0.52	0.52
Baltimore Laboratory—Oysters			
A. Alter	11.21	0.10	0.08
	11.18	0.12	0.10
H. I. Macomber	11.30	0.11	0.09
	11.22	0.10	0.09
H. S. Haller	11.16	0.09	0.08
	11.55	0.09	0.08

Collaborative results (Continued)

ANALYST	TOTAL SOLIDS	SODIUM CHLORIDE— BY OPEN CARIUS	SODIUM CHLORIDE— BY ASHING WITH CALCIUM ACETATE
	Scallops		
A. Alter*	19.29	0.31	0.29
	19.23	0.30	0.30
H. I. Macomber	19.52	0.34	0.34
	19.64	0.33	0.32
H. S. Haller	19.64	0.34	0.30
	19.61	0.32	0.32
	Boston Laboratory—Oysters		
J. A. Schuldiner	12.49	0.19	0.19
	12.47		
H. W. Haynes	12.43	0.19	0.19
	12.52	0.17	
J. J. Chap	12.47	0.17	0.18
	12.43		
C. H. Hickey	12.55	0.17	0.19
	12.43		
	Scallops		
J. A. Schuldiner	24.25	0.54	0.56
	24.38		
H. W. Haynes	24.48	0.54	0.57
	24.48	0.55	
J. J. Chap	24.45	0.52	0.55
	24.44		
C. H. Hickey	24.45	0.52	0.54
	24.29		

* Analysis made four days after sample was run by the other two analysts. Ground sample was held in refrigerator and was apparently in good condition.

Open Carius, and Sodium Chloride by Ashing with Calcium Acetate as Fixative, be adopted as tentative methods.

(2) That a study be made of methods compiled by P. B. Clark of the San Francisco Station of the Food and Drug Administration. A rough draft of these methods follows:

FISH

1. PREPARATION OF SAMPLE

To prevent loss of H_2O during preparation and subsequent handling do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in the following manner:

(a) *Fresh fish.*—With small samples remove one longitudinal half from each of 5–10 fish. In the case of large fish, cut from at least three fish three transverse slices, one inch thick; one slice from immediately back of the pectoral fins; one slice halfway between the first slice and the vent, and one slice immediately back of the vent.

Separate any bones that may be present as completely as possible from the pieces selected, leaving the skin intact so far as possible, since in many fish large quantities of fat are stored directly beneath the skin; pass rapidly through a food chopper three times, thoroughly mixing after each grinding, and begin all determinations as soon as practicable. If any delay occurs, chill the sample to inhibit decomposition.

2. TOTAL SOLIDS

Weight accurately 5–10 grams of sample, prepared as directed under **1**, into a tared lead dish containing ignited sand or asbestos fiber and a short glass rod. Distribute evenly over the bottom of the dish with a little water and place on a steam bath until the mixture appears dry. Heat to constant weight in a vacuum oven at 100° C., at a pressure not to exceed 100 mm. of Hg.

Report loss in weight as moisture.

3. ETHER EXTRACT

Place the entire dish and contents from the total solids determination in a Soxlet apparatus by rolling the dish to fit into the extraction thimble and extract for 16 hours with anhydrous ether. Evaporate the extract nearly to dryness on a steam bath and complete drying in the oven at the temperature of boiling water for 30 minutes. Cool in a desiccator and weigh. Continue, at 30 minute intervals, drying and weighing until the weight is constant.

4. ASH

Dry a sample representing about 2 grams of dry material and proceed as directed under XXVII, **8**, *Methods of Analysis, A.O.A.C.*, 1935, 336, at a temperature not to exceed 500° C. (In the case of material containing a large amount of fat, the preliminary ashing should be carried on at a sufficiently low temperature to allow smoking off the fat without burning.)

5. SODIUM CHLORIDE

See methods reeommended for adoption as tentative for oysters and scallops.

6. TOTAL NITROGEN—TENTATIVE

Proceed as directed under II., **19**, **22**, or **24**, using about 2 grams of the fresh sample.

Clark also furnished the following summary of comments received on these methods from discussions and correspondence with various chemists:

1. *Total Solids.*—Some collaborative work should be done to determine the advisability of drying at 70° C. or 100° C. in the air oven or vacuum oven. This work should possibly include an investigation of the effect of various methods of drying on the oxidation of the fat and the reproducibility of these methods so far as possible.
2. *Ether Extract.*—The same comments may apply to the drying of the ether extracts as to the drying for total solids. This work might well include determination of iodine number before and after drying.
3. *Ash.*—It has been suggested that the advisability of setting the temperature of ashing at "not to exceed 500° C." or "from 500° to 550° C." be discussed further.
4. *Chlorine.*—Collaborative work should be done to compare the two methods submitted with a view to determining whether one or both should be adopted.

Some of these methods may need collaborative study in different laboratories, but since they have been compiled from methods well recognized as accurate for other products, it may not be necessary to send out collaborative samples.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The work this year was limited to methods for the detection of added shell in cacao products, and the results are very encouraging. The study of lecithin anticipated last year was not made because no associate referee was appointed.

Some preliminary analyses of authentic samples of chocolate liquors by the crude cellulose method, *This Journal*, **19**, 415 (1936), showed that greater variation existed between the varieties than had been anticipated, due partly at least to difference in factory treatment, particularly roasting.

It was decided to use the limited time available for the investigation of the pectic substances, which had been determined previously, *This Journal*, **18**, 428 (1935), but the Referee did not believe the methods available were suitable for cacao products. Later work (unpublished) showed that the former figures were in error and that the ratio of pectic acid in shell to that in the nibs was greater than previously reported. A consideration of the function of pectic substances strengthens this conclusion. One of the principal functions of these substances in plant materials is to serve as a cement in the cell walls and as such they should be present in considerable amount in cacao shell. They are unnecessary for this purpose in the nibs.

The pectic acid results reported formerly (1.3% for nibs and 2.5% for shell) are now found to be in error. It appears that all the pectic material was not extracted from the shell by the solution used for this purpose. On the other hand, other substances (gums and nitrogenous material) were extracted from the nibs. They contaminated the pectic acid precipitate and gave high results. This contamination occurred whether the determination was made by the tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 324, **22**, or by the calcium pectate method, *Biochem. J.*, **16**, 60 (1922); **20**, 6 (1926). Thus high results on nibs were obtained by both methods.

It was found that practically all the soluble matter extracted by water and precipitated from shell is pectic material, while the nibs contain very little, their precipitate being composed of starch, gums, and nitrogenous

matter. Shell appears to contain considerable pectic material in the form of protopectin or insoluble free pectic acid.

It is well known that ammonium oxalate solution is an excellent solvent for the determination of total pectins.[1] The pectose is hydrolyzed by the solvent into its pectin and cellulose components. Considerably higher results were obtained by the use of this solvent than by extraction with water or dilute hydrochloric acid. A sample of shell gave 3.9 by the oxalate extraction, 2.3 by water extraction, and 3.0 by 0.0133 *N* hydrochloric acid extraction. Ammonium oxalate was therefore used in the final work as the most satisfactory solvent.

It was at first thought possible to separate pectinous material from the gums by partial precipitation with alcohol. The pectin in the shell extract required only between one and two volumes of alcohol while the gums required about four volumes of alcohol for complete precipitation. The method worked well on samples of pure shell or shell-free nibs, but on attempting to precipitate the pectin from samples of liquor containing 10–15 per cent shell, the Referee obtained very low results. Either the solubility of the pectin was not exceeded, or pectin was prevented from precipitating by the other substances present. The results, shown in Table 1, indicate that a separation of gums and pectin by this method is neither complete nor very reliable.

TABLE 1.—*Precipitation of pectin from samples containing varying amounts of shell*

SAMPLE	SHELL—FAT-FREE BASIS	PECTIN FROM 5 GRAM SAMPLE
	per cent	*mg.*
Liquor A	0.95	6.2
Liquor 2S	10.0	12.6
Liquor 43116	1.6	2.2
Shell X	100.	170.

The high results due to the presence of gums and other extraneous matter may be seen in Table 2. They were obtained by different treatment of duplicate samples.

TABLE 2.—*Results showing presence of gums and its effect upon the pectic acid figure*

SAMPLE	SHELL ON FAT-FREE BASIS	I SAMPLE EXTRACTED WITH ALCOHOL BEFORE EXTRACTION WITH AMMONIUM OXALATE. OXALATE EXTRACT ACIDIFIED TO pH 4.7 TO PPT. PROTEIN			II OXALATE EXTRACT ACIDIFIED TO pH 4.7, CENTRIFUGED, DECANTED AND TREATED WITH 10% TANNIC ACID
	per cent	*mg.*	*per cent*	*mg.*	*per cent*
Press cake	0.95	50.1	1.0	9.9	0.2
Liquor 2S	11.	51.3	1.03	38.4	0.77
Shell X	100.	220.7	4.41	2.02	4.04

[1] H W. Buston, *Biochem. J.*, 29, 200 (1935); Allen, Commercial Organic Analysis, X, p. 518.

These figures (Table 2) reveal that a large part of the material in the nibs that carried through and affected the final result was not pectin but other foreign material. The difference between the shell and nibs is seen to be much greater than was indicated before.

Other experiments to remove interferences were tried, trichloracetic acid and mixtures of trichloracetic acid and tannic acid being used. The filtration by the A.O.A.C. procedure continued to be sluggish and difficult in most cases regardless of whether the saponification was performed at low temperature or not. Finally, after it had been ascertained that when the pectic acid was first precipitated as calcium pectate and then converted into the pectic acid, the filtrations were rapid, and a procedure developed that gives good results. The method is as follows:

Place 5.0 grams of fat-free liquor or 16.0 of fat-free sweet chocolate in a 600 cc. beaker. Add 210 cc. of 0.5% ammonium oxalate, pouring in about 10 cc. and mixing to a paste before adding the bulk of the solution. Cover the beaker and place it on the steam bath for 3–3½ hours. Stir the mixture occasionally during the time it is on the bath. Remove, cool, stir, and transfer to a centrifuge bottle with the aid of a little water from a wash bottle and a policeman. Add 2.25 cc. of glacial acetic acid to the mixture, stopper, shake, and let stand for about 5 minutes. Centrifuge at 1800–2000 r.p.m. for 15–20 minutes. Carefully decant the supernatant liquid back into the beaker. Add a piece of domino sugar if the sample is a bitter liquor (about 4 grams). Add 100 cc. of 0.5% ammonium oxalate to the residue in the bottle, shake vigorously for a minute or two, add 1 cc. of glacial acetic acid, mix, and again centrifuge 15 minutes. Decant the liquid into the beaker containing the first extract.

Meanwhile boil the extracts down to a volume of about 125 cc. and cool; add 10 cc. of 10% tannic acid solution and allow to stand 10 minutes. Stir, and transfer the liquid to a centrifuge bottle with the aid of a minimum quantity of water, leaving no residue in the beaker. Centrifuge the mixture for 20 minutes. If the supernatant liquid is clear, carefully decant it into the original beaker. If the liquid is not clear, decant carefully into another centrifuge bottle, add 10 cc. more of tannic acid and centrifuge for 15 minutes, then decant the liquid carefully into the beaker whether clear or not.

Evaporate the liquid on the hot plate to 50 cc. (not below 40 cc.). Precipitate the pectin by adding gradually a mixture of 250 cc. of 96% alcohol plus 3 cc. of HCl (1+1). Allow the covered beaker to stand one-half hour and filter on an 18½ cm. C. S. & S. folded filter No. 588. Wash the residue into the filter with alcohol and allow to drain thoroughly. Take the filter from the funnel and spread it, and place the lower half in the beaker.

Dissolve and wash the residue back into the beaker with a stream of hot water from a wash bottle. Heat to boiling and filter if insoluble matter separates. Adjust the volume to about 90 cc. at room temperature and cool below 25° C. Add 10 cc. of 10% NaOH, mix, and let stand 20 minutes. Dilute the solution to 400 cc., add 3.5 cc. of glacial acetic acid, mix, and add 10 cc. of molar $CaCl_2$ solution with stirring. Allow the mixture to stand about 5 minutes and heat to boiling for a few minutes. Filter on an 18½ cm. C. S. & S. folded filter and wash the precipitate with hot water. Wash the precipitate back into the beaker with water and adjust to 100 cc. volume. Add 3.5 cc. of HCl (1+1) and heat to gentle boiling for 5 minutes. Filter on a 15 cm. paper, and wash with hot water. Transfer the pectic acid to a platinum dish, evaporate to dryness on the steam bath, and dry in the oven at 100° C. Weigh the dish and precipitate, ignite and re-weigh, and report as pectic acid.

Some results by the method presented are given in Table 3.

TABLE 3.—*Results obtained by the method presented*

SAMPLE	SHELL—FAT-FREE BASIS	PECTIC ACID, 5 GRAM SAMPLE	
	per cent	*mg.*	*per cent*
Liquor A	Less than 1	6.7, 6.0	0.13
Liquor 43110	1.33 or less	8.8, 10.9	0.18
Liquor 43104	1.10 or less	12.2	0.24
Liquor 43106	0.5	3.0	0.06
Liquor 2S	11	38.4	0.77
Liquor 3S	15	46.4	0.93
Press cake	About 1	12.5	0.25
Shell Z*	100	177.3	3.55
Shell X	100	194.4	3.89

* This shell sample was laboratory ground and particles were coarse.

The figures (Table 3) show plainly that pectic acid is an excellent index to the shell content of a sample. It appears that a result on pectic acid by the method given over 0.4 per cent would certainly indicate more shell than is usual with good factory process.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of pectic acid described in this report be subjected to collaborative study.

(2) That methods for the detection of added lecithin be further studied.

(3) That further collaborative work be done on the crude cellulose method for the determination of shell given in this report.

No formal report on gums in foods was given by the referee.

REPORT ON OILS, FATS AND WAXES

By G. S. JAMIESON (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the past year a collaborative study was made on the refractometric determination of the oil content of flaxseed under the direction of Associate Referee Lawrence Zeleny, Grain Division, Bureau of Agricultural Economics, who will present the report with recommendations. As H. Conway, North Dakota Regulatory Department, at Bismarck, was unable to serve as Associate Referee, collaborative work on methods for the determination of acetyl value and hydroxyl number was not continued (see 1935 report of W. L. Roberts). Also the Referee was unable to have work done, as authorized by the Association, on the Kaufmann thiocyanogen method.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 65 (1937).

A collaborative study was made on the colorimetric methods devised by J. Fitelson, New York Station, Food and Drug Administration, *This Journal*, **19**, 493 (1936), for the detection and the approximate determination of tea seed oil in admixture with olive oil, and the qualitative method of Wm. Siebenberg and W. S. Hubbard.[1]

The Fitelson method is based on the procedure of mixing carefully measured quantities of acetic anhydride, chloroform, and sulfuric acid, to which, after suitable cooling, is added a measured quantity of the oil to be tested, and holding the mixture at 25° C. for 5 minutes. The color is observed after dilution with 10 cc. of anhydrous ether. Tea seed oil gives a characteristic red color.

Samples of olive oil, refined tea seed oil, and two mixtures, labeled A and B, prepared from these oils, were sent to collaborators, some of whom had had no previous experience with the methods.

Sample A consisted of olive oil with 20 per cent tea seed oil and Sample B contained 40 per cent tea seed oil. The collaborators were asked to determine the amount of tea seed oil by the Fitelson method in Samples A and B and to use the pure oils submitted for the preparation of standards. For the purposes of this trial the collaborators were asked to estimate the quantity of tea seed oil in the samples to the nearest 5 per cent interval. The qualitative test by the Siebenberg-Hubbard procedure was also requested on each sample. The results are given in Table 1.

The Fitelson method gave no pink or red color with corn, cottonseed, mustard seed, peanut, sunflower, sesame, soybean, poppyseed, or rape seed oils.

Collaborators commented on the characteristic distinctive color the Fitelson method gave with tea seed oil alone or in mixture with olive oil, and that the colors given by the Siebenberg and Hubbard procedure could not be considered so distinctive, nor did they always appear to correspond with the description in the author's paper. The opinion is that the Siebenberg and Hubbard method as described is not generally satisfactory for the detection of tea seed oil both in regard to the colors obtained and the time required to make the tests.

In a private communication from C. S. Brinton, Chief of the U. S. Food and Drug Inspection Station, Philadelphia, Pa., results are reported demonstrating the accuracy of the Fitelson method in estimating the amount of tea seed oil present in olive oil. Two unknown samples were submitted by Brinton to a group of three chemists for determination of the amount of tea seed oil present. Two of the analysts had had experience with the Fitelson test and one (Berman) had not tried it previously. The analysts worked independently. Sample 1 contained 40 per cent of tea seed oil and Sample 2, 16 per cent in admixture with olive oil. The results (Table 2) show that the Fitelson test is accurate and confirm the

[1] *Oil and Soap*, **13**, 194 (1936).

TABLE 1.—*Collaborative results*

COLLABORATOR	SAMPLE	TEA SEED OIL FOUND	
		FITELSON METHOD	SIEBENBERG-HUBBARD METHOD
		per cent	
C. S. Ferguson	A	15–20	Doubtful
State Public Health Lab'y	B	35–40	Positive
Boston, Mass.			
L. W. Ferris	A	20	—
Food and Drug Administration	B	35–40	—
Buffalo Station			
F. J. Fitelson	A	20	Doubtful
Food and Drug Administration	B	40	Doubtful
New York Station			
H. J. Fisher	A	20	Positive
Conn. Agr. Expt. Station	B	35	Positive
New Haven			
F. L. Hart	A	15–20	
Food and Drug Administration	B	40	Positive
Buffalo Station			
R. L. Horst	A	20	Positive
Food and Drug Administration	B	40	Positive
Boston Station			
D. C. McLaren	A	15–20	Positive
Food and Drug Administration	B	40	Positive
Buffalo Station			
M. Tubis	A	Slightly less than 25	Positive
Food and Drug Administration	B	40	Positive
Philadelphia Station			
E. K. Tucker	A	20	Positive
Ala. Div. Agric. Chem.	B	35	Positive
Montgomery			
F. J. Vintinner	A	20	Positive
State Board of Health	B	40	Positive
Concord, N. H.			
C. A. Wood	A	20	Doubtful
Food and Drug Administration	B	40	Doubtful
New York Station			

TABLE 2.—*Additional results by Fitelson method*

ANALYST	TEA SEED OIL FOUND	
	SAMPLE 1	SAMPLE 2
	per cent	*per cent*
Edward O. Haenni	45	15
Manuel Tubis	40	15
Solomon Berman	35	15

experiences of the other workers that the estimation of the quantity present is more accurate with smaller than with larger amounts. The test gives concordant, reproducible results in the hands of various analysts, including those inexperienced with the method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the Fitelson method be made official (first action).

(2) That both the Malfatti and the Stout and Schuette methods for preparation of aldehyde-free alcoholic KOH be substituted for the present procedure (second action).

(3) That a collaborative study be made of methods for the determination of free fatty acids in both crude and refined fats and oils. The official method for the determination of free fatty acids, which was adopted many years ago, is no longer suitable for certain oils. Separate procedures are necessary for crude fats and oils, and for the refined products.

(4) That the refractometric method proposed for the determination of the oil content of flaxseed be made official (first action), and that a collaborative study be made of the application of the refractometric method to the analysis of one or more of the other commercially important oil seeds.

(5) That a collaborative study be made of the Kaufmann thiocyanogen method.

(6) That the revised specifications of the National Bureau of Standards for the titer thermometer to lengthen the thermometer to permit extension of the range to 66° C. be adopted as official (second action).

REPORT ON REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS

By LAWRENCE ZELENY (Bureau of Agricultural Economics, Washington, D. C.), *Associate Referee*

A need has long been felt for a method for the determination of the oil content of flaxseed that would be both accurate and sufficiently rapid for use in commercial inspection procedures. In an effort to establish such a procedure the refractometric method of Coleman and Fellows[2] was carefully investigated, as it was considered to be the most promising of various proposed rapid analytical methods. This method is based upon the fact that the refractive index of a mixture of halowax (α-chloronaphthalene) and a vegetable oil bears a linear relationship to the percentage of the oil in the mixture. This principle was first used by

[1] For report of Subcommittee C and action of the Association, see *This Journal* **20**, 61 (1937).
[2] U. S. Dept. Agr. Bull. 1471 (1927).

Wesson[1] in the determination of the oil content of cottonseed meal and meats.

In general, the method of Coleman and Fellows has given satisfactory results, but it is subject to a source of error arising from the variability of the refractive index of the oils from different lots of flaxseed. This variability has greatly increased during recent years, primarily because of unfavorable climatic conditions in the principal flax-growing areas. Means of eliminating this source of error, as well as other minor modifications, have therefore been incorporated in the proposed refractometric method.

Using a mixture of halowax and α-bromonaphthalene adjusted to a refractive index of 1.63940 at 25° C. as a standard solvent, the Associate Referee determined the refractive indices of mixtures of this solvent with various proportions of a composite sample of freshly prepared flaxseed oil, $n_D^{25} = 1.47780$ (Table 1).

TABLE 1.—*Refractive indices at 25°C. of known mixtures of the halowax, α-bromonaphthalene solvent with a petroleum ether extract of a composite sample of flaxseed*

OIL IN MIXTURE	n_D^{25}
per cent	
0.000	1.63940
4.877	1.62861
9.713	1.61853
11.537	1.61485
13.360	1.61114
15.610	1.60656
23.972	1.59062
100.000	1.47780

The percentage of oil in the mixture obtained in the actual analysis of the flaxseed may be calculated by the formula:

$$\frac{100\,Wx}{W' + Wx},$$

where W = weight of ground flaxseed (grams),
W' = weight of solvent (grams), and
x = weight of oil (grams) in 1 gram of flaxseed.

If this formula is used, it may be shown that a range in the oil content of flaxseed of 30–45 per cent will correspond to a range in the oil content of the solvent-oil mixture of approximately 10–15 per cent, when the recommended ratio of ground seed to solvent is used (2 grams of ground seed to 4 cc. of solvent). Over this range the oil content of the solvent-oil mixture bears an essentially linear relationship to the refractive index,

[1] *Cotton Oil Press*, **4**, 70 (1920).

each increment of 1 per cent in oil content corresponding to an increment of 0.002030 in refractive index at 25° C.

Thus the refractive index at 25° C. of the solvent-oil mixture may be calculated for any value of the oil content of the seed by the formula:

$$n_D^{25} = 1.61853 - 0.002030\left(\frac{100\,Wx}{W'+Wx} - 9.713\right).$$

Using this equation, the Associate Referee prepared a table, *This Journal*, **20,** 76 (1937), for converting refractive index readings of the halowax, α-bromonaphthalene extract into flaxseed oil content percentages. An auxiliary correction table has also been prepared, *Ibid.*, 77, by which corrections may be made for the variability of the refractive indices in oil from different samples of flaxseed.

COMPARISON OF THE REFRACTOMETRIC METHOD WITH THE PETROLEUM ETHER EXTRACTION METHOD

As a standard control procedure against which the results obtained by the refractometric method could be checked, the oil content of all samples was determined by extracting the finely ground seed for 20–22 hours with petroleum ether in some type of standard fat extraction apparatus. For this purpose a petroleum ether conforming to the official specifications for petroleum ether for cottonseed extraction was used.[1] In all cases the samples were reground in a mortar with carborundum or reagent-quality sea sand after the first four hours of extraction.

For the purpose of determining the accuracy of the proposed refractometric method, 84 samples of flaxseed were obtained from various sources, representing a great diversity of types and exhibiting a corresponding great diversity in physical and chemical characteristics. This assortment was represented by samples of the following types: (1) domestic commercial flaxseed; (2) Canadian commercial flaxseed; (3) Indian commercial flaxseed; (4) Argentine commercial flaxseed; (5) thirteen individual varieties; (6) samples grown experimentally in North Dakota, South Dakota, Minnesota, Kansas, California, Oregon, Wyoming, Arizona, Missouri, and New Jersey, and in the province of Saskatchewan; (7) immature flaxseed; (8) frost damaged flaxseed, and (9) scabby flaxseed.

The samples in this series showed the following ranges in physical and chemical characteristics: (1) Moisture content 4–16 per cent, (2) oil content 32.57–45.66 per cent (dry basis), (3) iodine number of oil (Wijs) 155.4–197.3, and (4) refractive index of oil at 25° C., 1.47589–1.48065.

These 84 samples were analyzed both by the petroleum ether extraction method and by the proposed refractometric method. These data, calculated to an 8 per cent moisture basis to conform to commercial practice, are listed in Table 2.

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[1] U. S. Dept. Agr. Service and Regulatory Announcement No. 133 (1932).

TABLE 2.—*Comparison of the oil content of 84 samples of flaxseed as determined by the petroleum ether extraction method and by the proposed refractometric method*

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD 8% MOISTURE BASIS	B PROPOSED REFRACTOMETRIC METHOD 8% MOISTURE BASIS	DIFFERENCE B−A
		per cent	*per cent*	*per cent*
108	Linota, 1935; Sheridan, Wyo.	29.96	30.03	+0.07
107	Redwing, 1935; Sheridan, Wyo.	31.02	31.04	+0.02
111	Bison, 1935; Newell, S. D.	32.15	32.07	−0.08
71	Linota, 1934; Fargo, N. D.	32.55	32.46	−0.09
40	Linota	32.67	32.83	+0.16
79	Linota, 1935; Moran, Kan.	33.00	32.79	−0.21
82	Linota, 1935; New Brunswick, N. J.	33.37	33.32	−0.05
32	N.D.R. 114	33.84	33.98	+0.14
72	Redwing, 1934; Fargo, N. D.	33.94	33.85	−0.09
73	Buda, 1934; Fargo, N. D.	34.08	34.21	+0.13
94	Commercial, musty	34.36	34.55	+0.19
39	Redwing	34.40	34.59	+0.19
80	Redwing, 1935; Moran, Kan.	34.77	34.87	+0.10
47	Abyssinian, 1935; Davis, Calif.	34.80	34.60	−0.20
33	Buda	34.86	35.13	+0.27
10	Domestic commercial	34.90	34.86	−0.04
81	Bison, 1935; Moran, Kan.	35.03	35.19	+0.16
74	Rio, 1935; Fargo, N. D.	35.14	35.12	−0.02
5	Bison, Missouri	35.15	35.32	+0.17
89	Domestic commercial, 1934	35.22	35.02	−0.20
35	Bison	35.22	35.26	+0.04
7	Bison	35.40	35.37	−0.03
88	Domestic comercial, 1934	35.47	35.50	+0.03
1	Bison; Arthur, N. D.	35.77	35.85	+0.08
66	No. 1 Canadian Western	35.79	35.57	−0.22
70	Bison, 1934; Fargo, N. D.	35.81	35.56	−0.25
90	Domestic commercial, 1934	35.87	35.73	−0.14
6	Domestic commercial, 1934	36.06	36.25	+0.19
31	New Golden	36.18	35.90	−0.28
84	Bison, 1935; Dickinson, N. D.	36.28	36.35	+0.07
8	Domestic commercial	36.37	36.66	+0.29
87	Domestic commercial, 1934	36.40	36.57	+0.17
68	No. 2 Canadian Western	36.51	36.53	+0.02
93	Domestic commercial, 1934	36.55	36.79	+0.24
62	Abyssinian, 1935; El Centro, Calif.	36.63	36.82	+0.19
48	Punjab, 1935; Shafter, Calif.	36.65	36.86	+0.21
51	Heavy frost damage, scabby	36.82	37.12	+0.30
67	No. 1 Canadian Western, 1934	36.86	36.88	+0.02
34	Walsh	36.91	36.88	−0.03
2	Bison	36.94	36.78	−0.16
3	Abyssinian, Calif.	36.99	36.94	−0.05

TABLE 2.—(*Continued*)

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD 8% MOISTURE BASIS	B PROPOSED REFRACTOMETRIC METHOD 8% MOISTURE BASIS	DIFFERENCE B—A
		per cent	*per cent*	*per cent*
49	Punjab, 1935; Madera, Calif.	37.02	36.82	−0.20
75	No. 3 Canadian Western	37.06	36.85	−0.21
37	Bolley's Golden	37.10	37.18	+0.08
50	Immature	37.15	37.09	−0.06
38	Rio	37.32	37.20	−0.12
83	Bison, 1935; New Brunswick, N. J.	37.32	37.39	+0.07
85	Bison, 1935; Morris, Minn.	37.34	37.45	+0.10
78	Rio, 1935; Moran, Kan.	37.36	37.33	−0.03
76	Argentine, imported commercial	37.41	37.25	−0.16
12	Argentine, imported commercial	37.46	37.51	+0.05
54	Punjab, 1935; Imperial, Calif.	37.46	37.53	+0.07
63	Abyssinian, 1935; Heber, Calif.	37.51	37.56	+0.05
96	Unknown	37.78	37.67	−0.11
46	Punjab, 1935; Davis, Calif.	37.86	38.12	+0.26
98	Unknown origin, 12.4% moisture	37.90	37.84	−0.06
99	Unknown origin	37.96	38.05	+0.09
86	Domestic commercial, 1934	37.97	37.91	−0.06
97	Unknown origin, 11.6% moisture	38.39	38.25	−0.14
69	No. 4 Canadian Western	38.47	38.40	−0.07
92	Domestic commercial, 1934	38.72	38.89	+0.17
56	Punjab, 1935; Holtville, Calif.	38.94	39.04	+0.10
91	Domestic commercial, 1934	39.09	39.04	−0.05
44	Punjab, 1935; Concord, Calif.	39.13	39.20	+0.07
45	Punjab, 1935; Rio Vista, Calif.	39 22	39.42	+0.20
57	Punjab, 1935; El Centro, Calif.	39.24	39.10	−0.14
64	Indian, 1934; Calexico, Calif.	39.27	39.39	+0.12
4	Punjab, Calif.	39.37	39.19	−0.18
53	Punjab, 1935; Holtville, Calif.	39.43	39.25	−0.18
42	Punjab, 1935; Davis, Calif.	39.51	39.39	−0.12
43	Punjab, 1935, Willows, Calif.	39.55	39.49	−0.06
77	Punjab, 1935; Yuma, Ariz.	39.67	39.50	−0.17
61	Punjab, 1935; Holtville, Calif.	39.70	39.85	+0.15
95	Unknown origin, 15.6% moisture	39.72	39.56	−0.16
55	Punjab, 1935; Brawley, Calif.	39.97	40.02	+0.05
58	Punjab, 1935; Calipatria, Calif.	40.06	40.13	+0.07
59	Indian, 1934; Brawley, Calif.	40.07	40.21	+0.14
41	Punjab, 1935; Davis, Calif.	40.30	40.00	−0.30
52	Punjab, 1935; Calexico, Calif.	40.37	40.18	−0.19
36	Punjab	40.46	40.61	+0.15
100	Unknown origin, 16.4% moisture	40.48	40.29	−0.19
65	Indian, 1934; Brawley, Calif.	40.68	40.83	+0.15
60	Indian, 1934; Holtville, Calif.	40.82	40.93	+0.11
11	Indian, imported commercial	42.01	41.84	−0.17

The coefficient of correlation between the oil content data as determined by the two methods is +.993, with a standard error of prediction of ±0.26 per cent oil. The errors in the refractometric method range from −0.30 per cent to +0.30 per cent oil on the basis of the control method, with an average error of ±0.13 per cent oil. Since the control method itself is subject to errors of at least half this magnitude, it appears that the proposed refractometric method yields results of the same order of accuracy as the petroleum ether extraction method.

COLLABORATIVE STUDY OF THE REFRACTOMETRIC METHOD

As a further means of checking the accuracy of the refractometric method, 10 samples of flaxseed, each representing a single variety, were

TABLE 3.—*Oil content of 10 samples of flaxseed, representing 10 individual varieties, as determined by 5 collaborators using the proposed refractometric method and the petroleum ether extraction method. All data are averages of duplicate determinations, and are expressed as per cent*

SAMPLE NUMBER	VARIETY	COLLABORATORS*					AVERAGE
		A	B	C	D	E	
				Modified Refractometric Method			
31	New Golden	36.65	37.07	36.89	36.83	36.56	36.80
32	N.D.R. 114	34.74	34.64	34.42	34.81	34.72	34.67
33	Buda	35.33	35.25	35.01	35.55	35.70	35.37
34	Walsh	37.73	37.62	37.63	37.95	37.56	37.70
35	Bison	36.03	36.00	36.05	35.90	35.72	35.94
36	Punjab	40.77	41.26	40.45	40.54	41.23	40.85
37	Bolley's Golden	37.60	37.95	37.74	37.16	37.90	37.67
38	Rio	37.75	38.78	38.16	37.69	37.85	38.05
39	Redwing	35.13	34.49	34.92	34.36	35.23	34.83
40	Linota	33.34	32.77	34.16	33.70	33.51	33.51
				Extraction Method			
31	New Golden	37.09	36.78	37.93	36.40	36.85	37.01
32	N.D.R. 114	34.98	34.41	35.77	34.18	34.57	34.78
33	Buda	35.71	35.26	36.78	35.21	35.43	35.68
34	Walsh	37.73	37.29	38.76	37.01	37.59	37.68
35	Bison	35.82	35.55	36.39	35.37	35.68	35.74
36	Punjab	40.51	41.24	40.49	41.17	41.08	40.90
37	Bolley's Golden	37.82	37.93	39.50	37.97	37.83	38.21
38	Rio	37.66	38.28	39.31	37.34	37.96	38.11
39	Redwing	35.20	35.01	36.41	34.61	35.03	35.25
40	Linota	33.41	33.53	34.83	33.32	33.34	33.69

* A. M. Agster, Spencer Kellogg & Sons, Inc., Buffalo, N. Y.
B. Leo F. Puhr, Department of Agronomy, South Dakota Agricultural Experiment Station, Brookings, S D.
C. W. F. Geddes, Grain Research Laboratory, Board of Grain Commissioners, Winnepeg, Can.
D. S. O Sorensen, Archer-Daniels-Midland Company, Minneapolis, Minn.
E. The Associate Referee.

analyzed by five collaborators. The results obtained, together with the results on the same series of samples obtained by the same collaborators by the petroleum ether extraction method are shown in Table 3. In each case the data were reported on a fresh weight basis in order that the additional variable involved in the determination of moisture might be avoided, as it is difficult to dry finely ground flaxseed without a significant increase in weight due to oxidation of the oil. Whereas some variation in the moisture content of the samples analyzed by the various laboratories may have occurred, such differences should be relatively small as all samples were distributed in moisture-proof metal containers.

Statistical analysis of the data in Table 3 yields the following results:

	PETROLEUM ETHER EXTRACTION METHOD	PROPOSED REFRACTOMETRIC METHOD
	per cent oil	*per cent oil*
Standard deviation between collaborators	1.66	0.19
Experimental error	.35	.33

Although the experimental error between the two methods does not differ significantly, the much lower standard deviation between laboratories in the case of the refractometric method suggests that this method should lead to more concordant results between different laboratories than will the extraction method when the analyses are carried out without central supervision.

As a result of the personal investigations of the Associate Referee and of collaborative tests, it may be concluded that the proposed refractometric method for determining the oil content of flaxseed is fully as accurate and reliable as the petroleum ether extraction method. The proposed method is much more rapid than the extraction method, a single determination requiring 20–30 minutes as compared with 16–24 hours required by most conventional extraction procedures.

RECOMMENDATIONS[1]

It is recommended—

(1) That the proposed refractometric method for the determination of the oil content of flaxseed be adopted as official (first action).

(2) That the possibilities of the method in relation to the analysis of other commercially important oil-bearing seeds be investigated.

No report on thiocyanogen value was given by the associate referee.

No formal report on microanalytical methods was given by the referee.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 61, 74 (1937).

REPORT ON MICROBIOLOGICAL METHODS—CANNED FOODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The report of the Referee on Microbiological Methods last year, *This Journal*, **19**, 428 (1936), contained a statement to the effect that culture media and procedures then suggested by the associate referees would be subjected to critical study during the year to test their suitability for inclusion as steps in proposed methods for the microbiological examination of canned foods. To some degree this statement constituted a vain promise, since for various reasons it has not been possible to conduct any such organized collaborative work. However, the year has not been wasted, since among the various associate referees there has been a continued exchange of opinion based on personal experience and upon the results of definite experimentation. As a result, full consideration has been given to the merits and demerits of the procedures recommended.

These procedures represent the best judgment of the several associate referees after careful and painstaking survey of the available information in this field. Nothing new regarding the several topics assigned for study has been disclosed as a result of inquiries during the year, and consequently, with the exception of one report, of which more will be said presently, there will be no report by any of the associate referees on microbiological methods this year. It is believed that nothing is to be gained by continuation of the investigation of the fundamental procedures heretofore listed for study, and therefore the Referee is recommending the abandonment of refereeships on these topics.

There have now been sufficient orientation and organization and enough clarification of thought in connection with methods for the microbiological examination of canned foods to permit the next logical step, which is the actual formulation of methods. A preliminary step in this direction was made last year when methods for the bacteriological examination of sugar were presented, *This Journal*, **19**, 439 (1936). It is proposed that these methods as described stand for another year for further trial and criticism. As an additional step, there is now presented for consideration a set of directions for the microbiological examination of one class of canned foods, namely, canned meats, on which E. J. Cameron will report. It will also be recommended that an associate referee be appointed to assume supervision over further work in that special field.

The course of action now indicated in the program calls for the appointment of associate referees on specific products, whose work will be the formulation of proposed methods as promptly as possible. Recourse will be had to the reports of last year and all counsel and assistance obtainable

will be utilized. Under the stewardship and supervision of each associate referee throughout the ensuing year these methods may be subjected to critical study and trial, and if they withstand the test, may be submitted as suggested procedures at the Annual Meeting next year. It is not presumed that under this procedure there can be built up a set of directions for the examination of any product that will be without fault at first reporting or that will not require amendment, but it is believed that a program of this nature will produce methods formulated upon sound principles for all bacteriologists working in the food field to test and criticize. Ultimately, and in the not too distant future, it will be possible to move for adoption of these methods and thus attain some part of the desired objective.

RECOMMENDATIONS[1]

Accordingly, the recommendations are as follows:

(1) Abandonment of the associate refereeships on the several topics assigned during the past year.

(2) Appointment of associate referees for the microbiological examination of (a) canned vegetables, (b) canned tomatoes and fruits, (c) canned fishery products, (d) canned meats, and (e) sugar.

No report on treatment of unopened container was given by the associate referee.

No report on sampling inoculum was given by the associate referee.

REPORT ON CULTURE MEDIA FOR NON-ACID PRODUCTS

METHODS FOR THE EXAMINATION OF CANNED MEATS

By E. J. Cameron (Research Laboratories, National Canners Association, Washington, D. C.), *Associate Referee*

Reports on microbiological methods submitted at the 1935 meeting of this Association dealt principally with elements of technic and culture media to be used in the examination of canned foods in general. When the general recommendations made are applied to certain specific classes of canned foods, however, it will be found necessary to make some modifications in treatment, and likewise when alternative procedures are suggested, to make a proper choice.

An illustration of the application of general procedures to a class of foods may be had in the following suggestions for the bacteriological

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 20, 62 (1937).

examination of canned meats. The methods suggested are the result of work conducted by M. L. Laing, F. W. Kurk, and F. T. Breidigan of the bacteriological sub-committee of the Technical Meat Committee of the National Canners Association, and they have been approved by the principal committee. They have been tested by meat technologists and are regarded as practicable from the standpoint of manipulative technic. They are also capable of producing essentially comparable results in the hands of experienced workers. These methods have not been tested by bacteriologists not immediately connected with the meat canning industry, but next year it is planned to solicit the aid of other food bacteriologists for the purpose of obtaining a wider range of opinion regarding their suitability.

Canned meat samples are commonly submitted to the laboratory for one of the following three purposes:

1. Unspoiled samples—for direct bacteriological examination for sterility.
2. Unspoiled samples—for examination as to keeping quality.
3. Spoiled samples—for examination for cause of spoilage.

The technic of examination for all three types of samples is similar with respect to the treatment of the container, the removal of the sample, and culture methods. Differences in treatment include the following:

(a) Before being cultured, unspoiled samples submitted for examination as to keeping quality should be incubated at 37.5° C. for a period of one month. This time of incubation, necessarily an arbitrary matter, is considered the longest period that is practicable in the usual case. Anaerobes, which at times may remain dormant for many months, may escape detection, but usually the likelihood of spoilage in the product under ordinary commercial conditions of handling is indicated in this time. It is regarded as unnecessary to incubate at thermophilic temperature, *i.e.*, 55° C., because such spoilage in meat products is extremely rare.

(b) Samples submitted for examination for cause of spoilage should be given direct microscopical examination in order to obtain general information regarding the bacterial flora. Ordinary laboratory stains, such as carbol fuchsin or gentian violet, are suitable in preparing mounts. The gram stain is not recommended. A gram negative result would be of little significance because of lack of knowledge regarding the age of the bacteria in the spoiled material.

The various steps in culturing technic are described in order as follows:

A. *Physical examination and preparation of the can:*

1. Note and record all marks of identification, either embossed on the can or appearing on the label.

2. Remove labels. Record any physical defects such as rustiness, pinholing, dents, improper closure, or defective side seams. Plainly mark for inspection questionable points if the can is to be pumped or given any other physical examination after it is opened.

3. Clean the container with soap and water; if it is greasy, it may be found helpful, especially at the site of opening, to apply a suitable solvent, such as petroleum ether, alcohol, or naphtha.

4. For sterilization at the site of opening, preferably grasp the container in the hand and hold the previously cleaned top in the flame of a Bunsen burner, distributing the heat with a circular motion. Do not play the burner down upon the top of the can because this will result in a concentration of heat at the top, causing scorching of the material, and it might lead to spurting of the contents when the opening is made. Such sterilization also causes a release of vacuum in the can, which will prevent any contamination that might result from an inrush of air when the opening is made. When containers are badly swollen, it may be unwise to flame in the manner described. In such instances apply the treatment described by Fellers, *This Journal*, **19**, 430, (1936), who suggests sterilization with bichloride of mercury (1+1000). As an alternative it is suggested that the cans be thoroughly cleaned with 60% alcohol. Whichever later treatment is used, first thoroughly cleanse the cans with soap and water, as it is possible that neither the bichloride nor the alcohol treatment would insure complete destruction of spore contaminants in the time that elapses between the sterilization treatment and the opening of the container. Thus mechanical removal of contamination should be regarded as an essential partial treatment.

B. *Removal of sample:*

1. *Opening of container.*—After flaming, or otherwise sterilizing the point of opening, make an aperture with an appropriate type of opener, which has also previously been sterilized by flaming. (Openers of the spiral or circular type, which cut a circular disk around a central puncture made by the instrument, are preferred, because those that make the cut at the can seam offer greater opportunity for the contamination that arises from excessive manipulation and greater surface exposure.) With liquid products, puncture an opening with a sharpened instrument of appropriate diameter. This subject has been discussed in detail by Tanner, *This Journal*, **19**, 432 (1936).

2. *Inoculum.*—As suggested by Tanner (*ibid.*), determine the type of instrument used for the removal of the inoculum by the character of the food under examination. Sample liquid or semi-liquid food products with sterile untapered pipets or inverted 10 cc. pipets. Sample solid material with sterile cork borers or brass sampling tubes after they have been wrapped in paper and sterilized for 30 minutes at 15 pounds in the autoclave. Stopper the samplers with cotton plugs, and force the solid material into culture tubes by means of a sterile glass rod or some similar device. As also suggested by Tanner, the sample should consist of at least 15 grams of food material, which may be cultured directly into one culture tube or flask, but preferably into at least three culture vessels. If the material is solid, mix it with sterile water as a preliminary step to inoculation.

3. *Sampling of product fractions.*—If it seems to be desirable to obtain inocula from component parts (fat-liquid-meat) of unspoiled canned meats, use special procedures based upon pre-incubation at a relatively high temperature. This has been done with roast beef in 24 ounce cans, corned beef in 6 pound soldered cans, and Vienna sausage in ½ pound cans.

Descriptive procedures are as follows:

Roast beef:

Separate roast beef into its component parts by pre-incubation at 50° C. for 24 hours. Draw off the liquid portion into a sterile glass-stoppered, wide-mouthed bottle, and place in the incubator to permit the fat to separate from the liquid portion. Pipet off 10 cc. of the separated fat layer and add to 25–30 cc. of a dispersing agent (2% gum tragacanth and 1 % gum arabic in water). Agitate the fat and dispersing agent well in a mechanical agitator and make inoculations. Pipet samples

of the liquid portion from underneath the fat layer and culture directly. After freeing the meat from all liquid, remove with a sterile cork borer and culture.

Corned beef:

Separate the fat by incubating at 50°C. for 2 days and then chilling immediately. (This results in the separation of a large amount of fat.) After chilling, open the cans at two points: (a) at the top, at which point a fat sample can be obtained, and (b) on the side at about the center, removing the sample of meat by a sterile cork borer. Add the fat to the dispersing agent and culture, but culture the meat directly.

Vienna sausage:

Prior to opening, incubate the cans 24 hours at 37° C. Remove liquid samples by means of sterile pipets inserted through an opening in the top of the can. After removing all liquid, mash the sausages in the same can and remove samples for culture.

C. *Culture media:*

In a meat product that is processed under pressure, understerilization usually results in the survival of spore-forming anaerobes—principally those of the putrefactive group. Thus anaerobic media are of special importance in the examination of canned meats. In addition, as a preliminary to plating and pure culture study, enrichment media are necessary to propagate non-sporing bacteria and aerobic or facultative anaerobic spore formers. The following media are recommended for the purposes indicated. Special media for thermophilic bacteria are not necessary.

(1) *Veal infusion broth.*—This medium is satisfactory both as an enrichment medium and as a propagator of anaerobes. It is prepared as a standardized, dehydrated product and is marketed under the name of "Bacto-veal Infusion Medium" by the Difco Laboratories, Inc., Detroit, Michigan. Because of its standardization, its use in this form is recommended.

(2) *Bacto-nutrient broth.*—Recommended as an alternative enrichment medium. This product is also manufactured in dehydrated form by the Difco Laboratories, Inc.

(3) *Liver broth and beef heart peptic digest.*—Recommended as an alternative anaerobic media. These products were described in a previous report of the Associate Referee, *This Journal*, **19**, 436 (1936).

As to culture methods for further study of the organisms isolated by enrichment, it is recommended that The Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists serve as a guide.

D. *Routine examination:*

It is recommended[1] that original cultures be incubated at 37.5° C. for 48–72 hours.

No report on culture media for acid products was given by the associate referee.

No report on incubation periods and temperatures for cultures was given by the associate referee.

No formal report on feeding stuffs was given by the referee.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **20**, 62 (1937).

REPORT ON FLUORINE IN FEEDING STUFFS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Recent studies on the toxicity of inorganic fluorine in animal feeds seem to indicate the need for developing suitable methods for determining fluorine in such materials.

In 1926, Cristiani and Gautier[1] reported that gases, evolved at aluminum plants using cryolite as a raw material, definitely increased the fluorine content of neighboring vegetation to a point where cattle, feeding on such vegetation, suffered ill effects.

Since 1931, when the connection between fluorine in drinking water and mottled enamel in the teeth of children was definitely established by Smith, Lantz and Smith,[2] various animal feeding experiments have been made with a view to studying the toxicity of small quantities of fluorine. Of interest are the following recent findings:

(a) In swine the feeding of fluorine, either as NaF or rock phosphate, reduces feed intake, average daily gains in weight, and feed utilization. A fluorine content of 0.03 per cent in the diet has been found deleterious to the bone structure. Damage to kidneys was noticed by Kick[3] from diets containing 1 per cent or more of rock phosphate.

The feeding of fluorine, whether in the form of rock phosphate, phosphatic limestone or treble superphosphate, has resulted in toxic effects.[3]

(b) Ingestion of fluorine by dairy cattle produces changes in liver, kidney, suprarenal gland, and heart. According to Phillips[4] gross fluorine toxicosis results in a disturbed osseous metabolism.

Typical symptoms of fluorine toxicosis were found by Phillips, Hart, and Bohstedt[5] with 0.625 per cent of raw rock phosphate in the diet. This is approximately equal to 0.02 per cent of fluorine. Feeding cattle on a grain mixture containing 1.25 per cent of rock phosphate reduced the milk production 20–25 per cent.[5]

(c) The "safe limit" for animal fluorine intake can not be fixed with the same degree of definiteness as for human beings. While apparently somewhat higher than for humans it is much lower than early experiments seem to indicate. Some workers place it well below 0.02 per cent fluorine.

The analytical problem of fluorine in feeding stuffs differs quantitatively rather than qualitatively from the problem where foods are concerned. The chief source of deleterious amounts of fluorine in feeds would be mineral supplements. Analyses of these materials themselves for fluorine should offer little or no difficulty with methods already available. The problem of isolation of fluorine from feed mixtures can be expected to offer greater difficulties.

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[1] *Ann. méd. légale criminol. police sci.*, 6, 336 (1926).
[2] Univ. of Arizona Coll. Agr Bull 32 (1931).
[3] Ohio Agr. Exp. Sta Bull 558 (1935).
[4] Unpublished. paper presented at meeting of Amer Dairy Science Assoc, June, 1934.
[5] Wisconsin Agr. Exp Sta. Research Bull. 123 (1934).

RECOMMENDATIONS[1]

It is recommended—

(1) That the work on fluorine in feeding stuffs be continued.

(2) That a survey be made of analytical methods suitable for determination of the element in mineral supplements to feed.

(3) That such methods when found be tried on fluorine in feed mixtures.

REPORT ON STOCK FEED ADULTERATION

By P. B. CURTIS (Purdue University Agricultural Experiment Station, LaFayette, Ind.), *Associate Referee*

During the past year the Associate Referee confined his efforts to the problem of devising a method for detecting rock phosphate in mineral feeds.

Two methods were investigated, first, that of applying the neutral ammonium citrate method, as used in the analysis of fertilizer materials, to phosphorus carriers commonly used in mineral feeds, and second, that of determining the fluorine content of mineral feeds.

The first method appears to have some merit when applied to mixtures containing relatively high percentages of rock phosphate while the second method seems to be more valuable in determining lower percentages of rock phosphate. It is the opinion of the Associate Referee that with additional work it may be possible to estimate quantitatively phosphate rock when added to commercial feeding stuffs, especially fish and meat products containing bone.

RECOMMENDATIONS[2]

It is recommended—

(1) That further study be given to the detection of mineral adulterants in feeds.

(2) That a study be made of methods for the detection of adulteration of condensed milk products.

(3) That a study be made of methods for the detection of adulteration of cod liver oil.

(4) That further study be given to the microanalytical detection of iodine in feeding stuffs.

No report on mineral mixed feeds was given by the associate referee.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 49 (1937).
[2] *Ibid.*

REPORT ON MOISTURE

By H. A. HALVORSON (Feed Laboratory, Department of Agriculture Dairy and Food, St. Paul, Minn.), *Associate Referee*

At the annual meeting in 1935, the Association approved the following recommendations of Subcommittee A: "That a committee be appointed to study the whole moisture question with the object of selecting one official vacuum method for all materials and correlating the other moisture methods as much as possible," and "That the chairman of the Committee on Moisture serve as the associate referee."

In conformity with this action the Associate Referee reviewed all reports on moisture published in *This Journal* since 1915. Several contributed papers in *This Journal* were also reviewed. These papers gave the results of many different studies with various moisture methods, including a comparison of the toluene distillation procedure with oven methods, comparison of results obtained by the vacuum oven method with those secured at higher temperatures in the electric air oven, the effect of temperature and diminished pressure on moisture determinations, and evidence of chemical changes produced by oven drying with heat when results are compared with those obtained by drying in vacuum desiccators over sulfuric acid without heat.

Collaborative work on moisture during the past 20 years has dealt largely with the following products: feedstuffs, dried fruits, cheese and other milk products, wheat flour, alimentary pastes, and bread and bakery goods. Little work has been reported during this period on the following materials, for which methods are provided in the first, second, or third editions of *Methods of Analysis:* Bordeaux mixtures, calcium arsenate, coal tar food colors, coffee, fertilizers, gelatin, insecticides, leathers, salt, soap, soils, and tea.

A survey of the data in referees' reports and other papers on moisture seems to furnish strong support for the following conclusions:

(1) It is not possible to arrive at the absolute moisture content of agricultural products. The only practical method consists in defining it to be the loss resulting from drying the product under definitely specified conditions of time, temperature, pressure, etc., since variations of these factors within certain limitations produce differences in the percentages of moisture.

(2) While the desirability of having a uniform vacuum oven method that may be used on all materials is recognized, practical considerations must be taken into account. Some substances require special treatment, at least in their preparation for drying, if not in the drying process. For example, certain products must be dried on porous material such as sand or asbestos to remove the major part of their water in the time specified; other materials contain volatile oils that require an indirect method involving removal of both moisture and volatile matter at a higher temperature than would be practical for the majority of biological products. Because some organizations prefer a method requiring less time and many laboratories are not

Comparison of present specifications with 1930 A.O.A.C. moisture methods

PRODUCT	OVEN 1930	OVEN PRESENT	TEMP (°C.) 1930	TEMP (°C.) PRESENT	TIME (HOURS) 1930	TIME (HOURS) PRESENT	MAX. PRESSURE 1930	MAX. PRESSURE PRESENT	DISHES 1930	DISHES PRESENT	SIZE OF SAMPLES 1930	SIZE OF SAMPLES PRESENT
							mm. Hg				*grams*	
Alimentary pastes or wheat flour (subsequent to 1930 bread and baked products also included)	vac	vac	98–100	98–100	5	5	25	25	55×15 mm slip-in cover	55×15 mm. slip-in cover	2	2
	air	air	130±3	130±3	1	1	760	760	metal with cover	metal with cover	2	2
Butter	not spec.	*	boiling H_2O	*	Hourly to cons. wt.	*	760	*	20 sq. cm.	*	1.5–2.5	*
Cacao products or grain and stock feeds[1]	vac.	vac.	boiling H_2O	70 just above vac. chamber	5 to cons. wt.	5 to cons. wt	100 or in dry H at 760	100	not spec.	60×15 mm slip-in cover	2	2
Optional method[10] for grain and stock feeds	air	air	135±2	135±2	2	2	760	760	Al with cover	60×15 mm. slip-in cover	2	2
Meat and meat products[2]												
Plants[3]												
Cheese (official)	vac.	*	boiling H_2O	*	4	*	100 in slow cur dried air	*	55×15 mm. slip-in cover	*	2–3	*
Sugars, sirups, molasses[4] and confectionery	not spec.	*	boiling H_2O	*	10 to cons. wt	*	760	*	Ni, Pt, or Al	*	2–5	*
On pumice	vac.	*	70	*	Every 2 to cons. wt.	*	100	*	60 mm. metallic	*	1 of air-dried matter	*
Honey and maple products on quartz sand	vac	*	70	*	Every 2 to cons. wt.	*	100	*	55×40 mm. with cover	*	1 of air-dried matter	*

[1] Distillation with toluene method and drying in H_2SO_4 vacuum desiccator without heat method, also official (1930) and (1936).
[2] Drying without heat feedstuffs method when dried sample is to be used for further determinations, otherwise feedstuffs vacuum oven method.
[3] Vacuum oven method or drying without heat over H_2SO_4, official for feedstuffs (1930).
[4] Densimetric, pycnometer, and refractometric methods also available for certain products in this group (1930).

Comparison of present specifications with 1930 A.O.A.C. moisture methods (Continued)

PRODUCT	OVEN 1930	OVEN PRESENT	TEMP. (°C) 1930	TEMP. (°C) PRESENT	TIME (HOURS) 1930	TIME (HOURS) PRESENT	MAX. PRESSURE 1930	MAX. PRESSURE PRESENT	DISHES 1930	DISHES PRESENT	SIZE OF SAMPLES 1930	SIZE OF SAMPLES PRESENT
							mm. Hg					
Dried and malted milk	vac.	*	boiling H_2O	*	5	*	100 in slow cur. dried air	*	55×15 mm. slip-in cover	*	1–1½	*
Dried fruits[5]	vac	vac.	70	70	12	6	100 in slow cur. dried air	100	8.5 cm. with covers	8.5 cm. with covers	5–10	5–10
Dried apples (tentative)	air	Dropped in 1933	boiling H_2O	Dropped in 1933	4	Dropped in 1933	760	Dropped in 1933	8.5 cm. with covers	Dropped in 1933	5–10	Dropped in 1933
Gelatin[6]	water jacket	*	boiling H_2O	*	6	*	760	*	55 mm. slip-in cover	*	2	*
Coffee[7] or tea	vac or air	*	boiling H_2O or 105–110	*	5 or 5	*	100 or 760	*	not spec. not spec.	*	5 or 5	*
Spices[8] and other condiments	air	*	110	*	to cons. wt	*	760	*	not spec.	*	2	*
Vegetables[9] and vegetable products												
Oils, fats and waxes[11]		vac.		20–25° above boiling pt H_2O		Hourly to cons wt.		100		shallow glass lipped 6–7×4 cm.		5±0.2

[5] With raisins and other fruits rich in sugar use 5 g sample moistened on 2 g. of fine asbestos, first drying on steam bath (1930), (1936).
[6] Cool covered dishes in H_2SO_4 vacuum desiccator (1930).
[7] Feedstuffs vacuum oven method (1930).
[8] From loss in weight by drying subtract quantity of volatile ether extract (determined separately) (1930).
[9] Drying without heat over H_2SO_4, official for feedstuffs (1930).
[10] Alternative method when subsequent fat determination is not to be made on sample
[11] Method for moisture and volatile matter made official (first action) in 1931, *This Journal*, **15**, 78 (1932).
* Reports of referees in *This Journal* since 1930 show no important change in the procedure given in *Methods of Analysis* (3rd edition, 1930).

equipped with vacuum oven apparatus, the universal adoption of a single low-temperature vacuum oven method is seemingly impossible.

(3) Coordination of the activities of investigators of moisture methods for agricultural materials appears to be very desirable. Based on work already accomplished a central committee can classify many food and feed products according to the most suitable moisture method. Furthermore, equipment can be standardized by adopting such a policy and thereby inconvenience and duplication of apparatus in control laboratories can be avoided.

The accompanying tabulation suggests inconsistencies that may be eliminated. For example, comparison of the size of moisture dishes specified in the present method for grain and stock feeds with the size of those used for alimentary pastes and wheat flour shows only a slight variation, which can easily be corrected. Undoubtedly, the temperatures used in the air oven procedures for these two classes of products can be brought into unison also. Examination of the table shows that the time of drying, temperature, and pressure adopted for some other products may likewise be made uniform.

That an active committee may correct another form of discrepancy is illustrated by the method for gelatin. In 1920, Subcommittee C recommended (in addition to two other procedures) the adoption of the vacuum oven method with drying at 70°. Neither the second nor the third edition of *Methods of Analysis* contains this provision, and no subsequent action by the Association appears to have discarded the procedure.

RECOMMENDATIONS[1]

It is recommended—

(1) That all proposed changes in the moisture methods of this Association be first considered and approved by the Committee on Moisture.

(2) That the Committee be given the privilege of assigning work for future investigations, the directors of which shall report to the Committee.

(3) That the work of correlating the moisture methods of the Association by the Committee and the Associate Referee be continued.

REPORT ON BIOLOGICAL METHODS FOR ASSAY OF VITAMIN D CARRIERS

By W. B. Griem (Wisconsin Department of Agriculture and Markets, Madison, Wis.), *Associate Referee*

The method for the assay of vitamin D carriers by the tentative chick test, *Methods of Analysis, A.O.A.C.*, 1935, 351, has proved its value in research as well as in feed control activities. The Associate Referee is

[1] For report and action of the Association, see *This Journal*, 20, 45 (1937).

led to make several recommendations for changes in the method. They are made to improve the procedure as seems justified by experience and research.

While it is believed at this time that the method is not ready for adoption as official, the Associate Referee thinks that there should be no hesitation in revising the method to keep it abreast with current knowledge in chick assay work. The recommended changes are the incorporation of a positive control, the option of composite ashing of the tibiae of the groups instead of individual ashing of the tibiae, the major change to a three-week instead of a four-week feeding period, and the inclusion of a statement on interpretation of results.

The value of controls in biological assay work is generally accepted. The tentative method provides for a negative control but not for a positive control. This deficiency is to be corrected by including in the procedure the feeding of one or more control groups fed the basal ration, so supplemented with one or more levels of an assayed cod liver oil that at least one group will receive a ration supplemented to produce normal calcification.

Group ashing of the tibiae as an alternative to the present individual ashing of the tibiae is recommended as a means of shortening the laboratory routine. From a review of laboratory data on individual ashing of tibiae, the Associate Referee had determined that there was no great variation to be expected in the two procedures. In these calculations individual bone and ash weights of groups were added, and the percentages of ash determined. The percentages so calculated showed a very close correlation to the averages of the individual percentages. The study was extended by experimental work in comparing opposite tibiae.

The results are given in Table 1. In this study the left tibiae were treated according to the tentative procedure, while the right tibiae of each group were handled as a unit. An assayed cod liver oil was used to supply the vitamin D. Cod liver oil and corn oil additions to the basal totaled one per cent for all groups. The approximate conformity of results is evident, and the justification of group ashing as a means of rapid control is obvious. The maximum variation in the average ash figures for opposite tibiae in the six groups shown is 0.7 per cent. This occurred in Lot 3, receiving a sub-optimum amount of vitamin D, and the ash percentage falls in the range where the largest variations in individual ash percentages occur.

The principal objection to the procedure is the absence of any information on the individual variations. If this detail is desired later or if there is a laboratory mishap there remain the opposite tibiae for reference. If this method is employed the opposite tibiae are roughly dissected and preserved in ethyl alcohol.

Knowledge of the response of chicks to administration of vitamin D

and of the normal rapid depletion of vitamin D reserves after hatching indicates that the four weeks feeding period of the tentative method may be shortened to three weeks. Kline, Elvehjem, and Halpin,[1] using the technic of the tentative A.O.A.C. method, at three weeks obtained a spread of 12 per cent in percentage ash between the basal and the group fed a ration adequately supplemented with vitamin D. Last year the

TABLE 1.—*Comparison of composite ashing and averaging of individual ashes of opposite tibiae of chicks*

LOT NO.	RATION	NO. OF BIRDS IN GROUP	AV. ASH OF MOISTURE AND FAT-FREE TIBIAE COMPOSITE ASHING	AV. ASH OF MOISTURE AND FAT-FREE TIBIAE INDIVIDUAL ASHING
			per cent	*per cent*
1	A.O.A.C. Basal. No added Vitamin D	10	35.0	35.5
2	A.O.A.C. Basal. No added Vitamin D	11	34.1	33.6
3	A.O.A.C. Basal+Approx. 13.5 U.S.P. units of Vitamin D per 100 grams of ration	11	40.4	39.7
4	A.O.A.C. Basal+Approx. 13.5 U.S.P. units of Vitamin D per 100 grams of ration	10	38.5	38.7
5	A.O.A.C. Basal+Approx. 54 U.S.P units of Vitamin D per 100 grams of ration	10	46.9	46.3
6	A.O.A.C. Basal+Approx. 54 U.S.P. units of Vitamin D per 100 grams of ration	11	46.6	46.4

Associate Referee showed in an experiment that the birds receiving the basal ration were as severely rachitic at the end of the third week as they were at the end of the fourth week, *This Journal*, **19**, 585 (1936). An interpretation of the data then presented indicates that a three-weeks test could be substituted for the four-weeks test. After an exhaustive study of the subject, Lachat and Halvorson, *Ibid.*, **19**, 637 (1936), conclude that the length of the feeding period of the tentative method may be shortened from four weeks to three weeks and possibly even to two weeks. In the absence of any conflicting evidence these findings indicate that the procedure may safely be shortened. The reduced feed and labor cost, together with the more rapid completion of assay, makes this change desirable.

The present tentative method does not in any way provide for interpretation of results. In feed control activities it has chiefly been used to verify percentages at which vitamin D carriers produced complete pro-

[1] *Poultry Sci.*, **14**, 116 (1935).

tection against rickets, as measured by a minimum of 44 or 45 per cent average ash of the moisture and fat-free tibiae.

The method does not need to be so limited in its application. It is possible to interpret sub-optimum calcifications with a considerable degree of accuracy. For interpretation of such results comparisons of ash averages must be made with those produced by sub-optimum additions of vitamin D furnished by a standard. The accepted standard for vitamin D control work in pharmaceuticals is the U.S.P. Pharmacopoeia reference cod liver oil, containing 95 U.S.P. units of vitamin D per gram. It can be used in chick assay work as well. Through its use definite additions of vitamin D can be made to control rations. Comparative responses in calcification by products under assay can therefore be interpreted directly into comparative potencies.

Table 2 shows the results in calcification produced in two series of assays when the U. S. Pharmacopoeia reference cod liver oil was used as the control. Results of assay of four selected cod liver oils from each series are included. Series 1 was terminated March 18, 1936, and Series 2 on August 17, 1936. Cod liver oil and corn oil additions to the basal ration totaled one per cent for all groups. The tentative A.O.A.C. method with the four-week feeding period was used.

The progressive increases in calcification due to increasing additions of vitamin D are revealed. Lots 3 and 4, Series 1, are the only exceptions at sub-optimum levels of calcification. It must be remembered that the increases of 2.5 U.S.P. units of vitamin D per 100 grams of ration are small. The birds in Series 1 show a higher requirement for vitamin D than those in Series 2. Only 17.5 U.S.P. units per 100 grams of ration were required to produce a minimum of 44 per cent average ash in Series 2, compared with 22.5 units in Series 1. These differences stress the need for controls in every series of assays. The resultant ash averages of the assayed cod liver oils as shown in the data are readily interpreted into comparative potencies when based on the ash averages produced by the reference oil. In Series 1, Sample A at 20/95 per cent produced a calcification equal to that produced by the reference oil at the same level. It is therefore as effective as the control oil and contains approximately 95 comparative U.S.P. units of vitamin D per gram.

Sample B, claimed to contain 200 U.S.P. units of vitamin D per gram, at 20/95 per cent produced a calcification equivalent to that produced by 10 U.S.P. units from the reference oil and therefore contains approximately only 47.5 comparative U.S.P. units of vitamin D per gram. Sample C, claimed to contain 150 U.S.P. units of vitamin D per gram, and also claimed to produce complete protection against rickets at ½ of 1 per cent by the tentative A.O.A.C. method, produced a smaller average ash at this percentage than that produced by 10 U.S.P. units from the reference oil. The potency therefore is less than 20 comparative U.S.P.

units per gram, inasmuch as ½ gram contained less than 10 comparative U.S.P. units. Sample D, claimed to contain 95 U.S.P. units of vitamin D per gram, produced a calcification at 20/95 per cent that was superior to that produced by the reference oil at the same level. It is therefore

TABLE 2.—*Calcification responses in two series of assays by A.O.A.C. tentative method*

LOT NO.	RATION	SERIES I		SERIES II	
		NO. OF BIRDS IN GROUP	AV. ASH OF TIBIAE	NO. OF BIRDS IN GROUP	AV. ASH OF TIBIAE
			per cent		*per cent*
1	A.O.A.C. Basal. No added vitamin D	9	35.71	10	35.27
2	Basal+10/95% U.S.P. Reference C.L. Oil, 10 U.S.P. units per 100 grams	10	38.26	12	41.12
3	Basal+15/95% U.S.P. Reference C.L. Oil, 15 U.S.P. units per 100 grams	11	42.72	11	43.69
4	Basal+17.5/95% U.S.P. Reference C.O. Oil, 17.5 U.S.P. units per 100 grams	11	41.82	12	44.12
5	Basal+20/95% U.S.P. Reference C.L. Oil, 20 U.S.P. units per 100 grams	11	43.25	12	46.69
6	Basal+22.5/95% U.S.P. Reference C.L. Oil, 22.5 U.S.P. units per 100 grams	10	45.44	11	45.94
7	Basal+25/95% U.S.P. Reference C.L. Oil, 25 U.S.P. units per 100 grams	10	45.56	8	45.75
8	Basal+20/95% C.L. Oil A	11	43.36	—	—
9	Basal+20/95% C. L. Oil B	11	38.72	—	—
10	Basal+1/2% C. L. Oil C	11	36.50	—	—
11	Basal+20/95% C. L. Oil D	12	45.43	—	—
12	Basal+1/4% C. L. Oil E	—	—	10	37.78
13	Basal+1/2% C. L. Oil F	—	—	11	43.05
14	Basal+1/2% C. L. Oil G	—	—	9	42.66
15	Basal+1/2% C. L. Oil H	—	—	10	44.78

more effective than the U.S.P. reference oil and contains a minimum of 95 comparative U.S.P. units of vitamin D per gram.

In Series 2, by the same methods of interpretation, sample E contains less than 40 comparative U.S.P. units of vitamin D per gram; sample F, approximately 30 comparative U.S.P. units of vitamin D per gram; sample G, less than 30 but more than 20 comparative U.S.P. units of vitamin D per gram; and sample H, a minimum of 35 comparative U.S.P.

units of vitamin D per gram. The term "comparative U.S.P. unit" is used here to express in numerical value the response of vitamin D on the chick when compared to definite administrations of vitamin D from the U. S. Pharmacopoeia reference cod liver oil. The stated comparative values for the assayed oils are not actual U.S.P. units that can be determined only by the accepted rat assay procedure.

No attempt was made to carry out collaborative work in view of the impending recommendations for changes in the method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the second sentence of the tentative procedure, sec. 56, p. 351, *Methods of Analysis, A.O.A.C.*, 1935, be changed to read as follows: "Reserve one group for negative control purposes, one or more groups for positive control purposes, and one or more additional groups for each material to be assayed."

(2) That the parenthetical phrase in the first sentence of the second paragraph of the same procedure be changed to read: "(60 lbs. per 100 birds is ample)."

(3) That there be inserted, following the fourth sentence of the second paragraph of the same procedure, the following: "Supplement the basal ration with one or more levels of an assayed cod liver oil, the level or levels so selected that the birds of one group will produce normal calcification. (These are the rations to be fed to the positive control group or groups.)"

(4) That the second sentence of the third paragraph of the same procedure be changed to read as follows: "Beginning the third day feed the rations *ad libitum* for 21 days."

(5) That there be inserted after the second sentence of the fourth paragraph of the same procedure the following: "If composite ashing of the tibiae of the groups is to be made, preserve the opposite tibiae in ethyl alcohol."

(6) That the word "individually" be deleted from the fourth sentence of the fourth paragraph of the same procedure so that the sentence will read as follows: "Crush, wrap in filter paper, and extract the bones for 20 hours with hot 95 per cent ethyl alcohol, followed by 20 hours with ethyl ether."

(7) That the seventh sentence of the fourth paragraph of the same procedure be changed to read as follows: "Determine the percentage of ash of the moisture and fat-free bones by igniting individually or by group composite in a muffle furnace at approximately 850° for one hour."

(8) That there be added a new paragraph to the same procedure to read as follows: "To obtain vitamin D [or anti-rachitic] potency of a

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 49, 72 (1937).

product in terms of A.O.A.C. chick units, use the U. S. Pharmacopoeia reference cod liver oil in control rations at properly selected levels and compare the resulting control ash averages with that produced by the product under assay. One A.O.A.C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U.S.P. reference cod liver oil in this method of assay."

(9) That collaborative assay of vitamin D carriers be made and study continued on the tentative method as well as on the determination of the anti-rachitic potencies of proprietary feeds.

REPORT ON HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS

By ROBERT A. GREENE, *Associate Referee*, and EDWARD L. BREAZEALE
(Arizona State Laboratory, Tucson, Ariz.)

In accordance with the recommendations of the Association last year, *This Journal*, **19**, 47 (1936), that further study be given to the methods proposed by the Associate Referee, a sample of linseed meal was sent to the following collaborators:

(1) J. C. Kistler, Armour and Co., Chicago, Ill.
(2) C. Perry Coleman, Asst. State Chemist, Tallahassee, Fla.
(3) George E. Grattan, Department of Agriculture, Ottawa, Canada.
(4) Eugene F. Boyce, Agricultural Experiment Station, Burlington, Vt.
(5) A. P. Kerr, Department of Agriculture, Baton Rouge, La.
(6) George H. Marsh, Department of Agriculture & Industries, Montgomery, Ala.
(7) C. L. Hare, State Chemist, Auburn, Ala.
(8) E. L. Breazeale, Arizona State Laboratory, Tucson, Ariz.
(9) R. A. Greene, Arizona State Laboratory, Tucson, Ariz.

The collaborators were requested to determine hydrocyanic acid in the sample by the modified alkaline titration method, *Ibid.*, 94, using the method as given, and also using the indicator solution as recommended by Bartholomew and Raby, *Ibid.*, 474. They were also requested, if convenient, to use the Prussian blue method, *Methods of Analysis, A.O.A.C.*, 1930, 287, the colorimetric method of Frances and Connell,[1] and the qualitative test for the detection of cyanogenetic glucosides suggested by the Associate Referee, *This Journal*, **19**, 94 (1936). It was also suggested that if equipment were available, determinations be made with a photoelectric colorimeter, according to the method of Bartholomew and Raby.[2]

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[1] *J. Am. Chem. Soc.*, **35**, 1624 (1913).
[2] *Ind. Eng. Chem. Anal. Ed.*, **7**, 68 (1935).

The results of the determinations are given in Table 1.

TABLE 1.—*Hydrocyanic acid in sample of linseed meal*
(Results expressed as mg. of HCN per 100 grams of sample)

COLLABORATOR	MODIFIED ALKALINE TITRATION[1] (A)	(B)	PRUSSIAN BLUE	FRANCES AND CONNELL	QUALITATIVE
1	42.40	42.40	unsatisfactory	21.54	not made
2	33.44	33.44	not made	9.50	positive
3	40.50 32.40	40.50 32.40	59.09	24.88	positive
4	27.33	26.53	not made	not made	not made
5	44.79	42.68	30.28	30.60	not made
6	35.93	34.48	34.24	10 88	positive
7	35.19	35.19	unsatisfactory	unsatisfactory	not made
8	43.20	43.80	not made	not made	positive
9	44.00	44.00	unsatisfactory	33.00	positive
Average	37.92	37.54	41.20	21.23	

[1] Method "A" is the use of KI and NH_4OH solutions as recommended Method "B" uses the mixed indicator solution suggested by Bartholomew and Raby. (1 ml. of a solution, 5 grams of KI, 5 ml. of NH_4OH, and 95 ml of H_2O)

The results show that as far as the alkaline titration method is concerned, there is little difference whether the potassium iodide and ammonium hydroxide are added separately, or in the form of the indicator solution. It is the opinion of several of the collaborators and of the Associate Referee that the use of the indicator solution does not give quite as sharp an endpoint as when the solutions are added separately. In the latter case, larger amounts of ammonia and potassium iodide are added, which may account for the difference.

It was the general opinion of the collaborators that the Prussian blue method is unsatisfactory. When results were obtained, they varied considerably from those secured by the alkaline titration method. In the opinion of the Associate Referee, it is unsatisfactory. The principal difficulty of this method is in securing a suspension of Prussian blue which does not settle rapidly. This, as well as certain technical difficulties, should eliminate it from further consideration.

The results of the colorimetric method of Frances and Connell were equally variable, although they were uniformly lower than the results secured by the alkaline titration method. This is probably due to an incomplete conversion of sodium cyanide to sodium sulfocyanate. It is also difficult to entirely remove the excess of free sulfur.

Since the results of the colorimetric methods showed considerable variation, it is questionable whether they should receive further study. Kistler stated: "The colorimetric method, in my opinion, has nothing to recommend it. The long manipulation offers many chances of error."

Photoelectric turbidimeters were not at the disposal of any of the collaborators, so there was no opportunity to test this method. It has given excellent results in the hands of Bartholomew and Raby, and results secured by them last year on a referee sample agreed well with those secured by several collaborators who used the modified alkaline titration method. The Associate Referee believes that this is the most accurate method and it is recommended when the necessary equipment is available.

The qualitative test gave satisfactory results. It has been widely used and has always given satisfactory results. It is very valuable for preliminary tests to show the presence of cyanogenetic glucosides.

There is considerable variation in the results obtained by the collaborators. The Associate Referee believes that this does not detract from the method, but that it was caused by a loss of hydrocyanic acid from the sample during shipment or during storage before the examinations were made. Furthermore, due to the small amounts of hydrocyanic acid in the aliquot parts titrated, small variations in the amount of silver nitrate used, due to individual differences in judging the end point, and slight variations in burets, the differences are magnified when the results are expressed in terms of milligrams per 100 grams of sample. If a ten gram sample is used, the distillate made to a volume of 250 cc., and a 100 cc. aliquot part titrated, when the final results are expressed on the basis of 100 grams, all errors are magnified 25 times. Thus, in a 10 gram sample, an error in titration of 0.05 ml. will change the final results by 1.35 mg.

Although the end point is very sharp, it is necessary that the titrations be made against a black background in order to avoid over titrating. The end point may be easily recognized if the beaker in which the titration is made is illuminated by a beam of light which passes obliquely through the solution.

The samples were sent out in light card-board containers and it is quite possible that moisture might have come in contact with them, and that loss of hydrocyanic acid might have occurred. This may be illustrated by the experience of Coleman. A sample was mailed on August 25; he reported that the sample arrived in a moist condition and that he could not detect hydrocyanic acid by qualitative or quantitative methods. Accordingly, another sample was sent to him. His results were somewhat lower than those secured in Arizona. Grattan reported that the first results obtained were 40.5 mg. hydrocyanic acid; later they were 32.4. This may be due also to a loss of hydrocyanic acid.

This idea is supported by the following experiment: A sample of linseed meal was placed in one of the card-board containers used for mailing the samples, and the container was placed in an ice box. Determinations of hydrocyanic acid at frequent intervals showed a progressive decrease.

In spite of the variations in results, the modified alkaline titration method was the most satisfactory, and each collaborator was able to secure satisfactory checks. The probable cause of variations in the results of the several collaborators has already been given. It should be pointed out that the results of different collaborators showed better agreement than in previous years, when other methods were employed.

The authors gratefully acknowledge the assistance and splendid co-operation of the collaborators.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modified alkaline titration method suggested by the Associate Referee be adopted as official.

(2) That the use of an indicator solution, as suggested by Bartholomew and Raby, be made an optional part of this method.

(3) That the photoelectric tubidimetric method of Bartholomew and Raby be adopted as official.

(4) That the qualitative test for cyanogenetic glucosides in feeds and similar materials suggested by the Referee be adopted as official (first action).

(5) That the present tentative Prussian blue method be deleted.

(6) That since present colorimetric methods involve considerable manipulation, and have no advantages over the titration method, no further study be given to such methods.

(7) That if additional methods are desired, the turbidimetric methods of Fox[2] or Roe,[3] and the nesslerization method of Gale and Pensa[4] be studied.

REPORT ON FAT IN FISH MEAL

By R. W. Harrison (U. S. Bureau of Fisheries, Seattle, Wash.), *Associate Referee*

Fish meals vary considerably in fat content, depending upon the type of raw material and the method of processing used in their manufacture. Likewise consumer opinion differs materially as to the amount of fat a fish meal should contain. Accordingly the determination of fat in these products is an important consideration from a feed control standpoint.

The ordinary proximate analyses of fish meals made in feed or commercial testing laboratories, while not accurate indications of quality, are supposed to be indicative of general composition, and in a sense are

[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 49 (1937).
[2] *Science*, **79**, 37 (1936).
[3] *J. Biol. Chem.*, **58**, 667 (1924).
[4] *J. Ind. Eng. Chem. Anal. Ed.*, **5**, 80–1 (1933).

expected to give a fair indication of the nature of the product in question. Thus, when a testing laboratory reports a meal containing 70 per cent protein ($N \times 6.25$) as compared to another as containing 50 per cent protein ($N \times 6.25$) the buyer can assume with reasonable assurance that the former meal contains a larger amount of protein per unit of weight and approximately in proportion to the data reported. Unfortunately, however, the same cannot be said for a reported analysis for fat. This is because the extraction of fat is as yet an arbitrary determination dependent upon the solvent used and the nature and condition of the fat present in the meal.

Because of the confusion that has resulted from the uncertainty of fat determinations as applied to fish meals, both the U. S. Bureau of Fisheries and the Association of Official Agricultural Chemists have been asked to study the problem in an effort to determine, if possible, a method of procedure which will fit more closely the needs of those concerned with the manufacture, distribution, and consumption of this product.

It may be recognized that some of the present confusion is due to the variation in the action of the several fat solvents now being used in testing laboratories. The two most common solvents are ethyl ether and petroleum ether. Ethyl ether may vary in quality from a neutral anhydrous product, as prescribed by the Association of Official Agricultural Chemists, to relatively impure technical grades. Petroleum ether, not being a definite compound, also will vary in composition. Experience has shown that the two types of solvents give widely divergent results. An impure ethyl ether generally gives a higher value than a neutral anhydrous product, while petroleum ethers sometimes give results as much as 25 per cent lower than ethyl ether. It is apparent, therefore, where limits are placed on fat content, that present practices lead to a most unsatisfactory situation, especially in borderline cases.

Since solvents differ in their solvent action, uniformity of results will of necessity require uniformity of procedure, both from the standpoint of solvent and technic. The task confronting those interested in the solution of the problem is to determine the solvent which will come nearest to being specific for the purpose for which it is intended.

Fish meals are generally manufactured during a relatively short fishing season. Consumption, however, is reasonably uniform throughout the year. Accordingly, the greater part of the fish meal consumed must be held in storage for periods of a few months up to a year or more, depending upon market conditions.

Fish oils are known to be quite highly unsaturated and readily susceptible to oxidation. It is not surprising, therefore, that the fat in fish meal will experience considerable oxidative change during storage and in so doing become less soluble in the type of fat solvents now used. The writer has followed the change in ether extract values of fish meals

in storage and has observed a decrease of as much as 50 per cent over a period of nine months. The failure of ethyl ether and petroleum ether to meet this condition represents an added and perhaps the most significant difficulty of obtaining a true fat analysis of a fish meal by existing methods and suggests also the limitation of the present official A.O.A.C. method as pertaining to fat analyses of these products.

If the purchase price of a fish meal is in part predicted upon its fat content, those concerned with the purchase and sale of the product should have a means of knowing the true amount of fat present. It is the belief of the writer that where a consumer prefers lower fat content meals, his reasons are based not so much on adverse results caused directly by natural fresh oil but because such oils become oxidized and give stronger flavor and odors. There is need for biological studies of these highly oxidized oils to determine their effect on nutritional value and possible injurious effect. Thus, under present methods of testing, ether extract values may lead to a twofold deception because they do not indicate the actual amount of oil present in the meal and further give no measure of the condition the consumer is trying to eliminate when specifying a relatively low fat content meal. A solvent to be specific, therefore, must extract all fat present regardless of its condition.

In the work contemplated it is proposed to study a variety of solvents available for this purpose and to evaluate them according to the degree with which they approach the prescribed specification. Some difficulty is anticipated in interpreting results since it is expected that the various solvents will differ in their degree of extraction of both the fatty and nonfatty components of the meal. In order to reduce the possibility of confusion in interpreting data involving such variations, it is planned to carry out the work in the following general manner:

Both meal and oil will be prepared from the same lot of raw material. The meal will be dried under reduced pressure in the absence of oxygen. A portion of the meal will be held in an inert atmosphere for the purpose of constituting the initial tests, while the remainder will be subjected to normal exposure to air. At the same time, different lots of some absorbent material, which have been previously extracted with the various solvents to be studied, will be impregnated with a known amount of oil. These materials and the meals will be extracted at intervals over an extended period of exposure to air and the nature of both types of extracts investigated.

While it is realized that the impregnated material should be more easily extracted than the regular meal, it is felt these samples will be of value in indicating directly the solubility of oxidized glycerides and also give a better opportunity to study the changes in their characteristics. With these data as a check the writer hopes to be in a better position to interpret the results obtained from the regular meal. Data on the above program of study will be reported at the next meeting of the Association.

In concluding, the writer would like to suggest that the problem of

determining fat in fish meals no doubt also applies to those vegetable meals containing highly unsaturated fats.

No formal report on biological methods for vitamin B complexes was given by the associate referee. It was recommended[1] that additional studies be made on the proposed method before it is recommended as a tentative method, and that the investigations on the proposed method be continued and extended.

REPORT ON TECHNIC AND DETAILS OF BIOLOGICAL METHODS, VITAMIN D CARRIERS

By LAWRENCE LYSLE LACHAT[2] (Feed Laboratory, Department of Agriculture, Dairy and Food, St. Paul, Minn.), *Associate Referee*

During the past year considerable progress was made in ascertaining the extent of error in ash determination of chick tibiae with the procedure developed and recently described by the Associate Referee, *This Journal*, **18**, 357 (1935); **19**, 598 (1936). This year the work was planned to study the error that exists (1) in the dissection, cleaning, and preparation of the bone sample for subsequent extraction of crude fat and estimation of ash; (2) when several different types of solvent are employed for extracting crude fat; and (3) in evaluating comparative criteria for interpreting results of vitamin D bioassay.

PREPARATION OF BONE FOR ASH DETERMINATION

Many tests for vitamin activity have been made in this laboratory upon various substances containing vitamin D (and characterized by their adaptability to the A.O.A.C. method of bioassay) by the feeding technic developed by Minnesota,[3] by Wisconsin, and by Maryland. Because of these vitamin-testing experiments a large number of birds were available for further study of factors affecting accuracy of ash estimation and of various other criteria for interpretation of results. Carefully selected tibiae (left) from birds four weeks of age were used for analysis, the procedure for preparation, extraction, and ashing being the same as that described for the Association two years ago.

At the same time, the right tibia or opposite pair saved for collaborative study in conjunction with the corroborative work from other laboratories was treated in this way: Half the number from any one lot

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, **20**, 50 (1937).
[2] Present address: The Great Atlantic and Pacific Tea Co., Minneapolis, Minn.
[3] *Poultry Sci.*, **15**, 127 (1936).

of birds were dissected, cleaned, and prepared for the ash estimation by the same method used on the left tibia prior to extraction of crude fat. These, labeled "prepared specimens" for convenience, were preserved individually in vials containing 95 per cent ethanol, and subsequently these cleaned bones were forwarded to the collaborator. The remaining half, left unprepared but severed cleanly and removed completely from the body of the birds, were promptly frozen, labeled "frozen specimens," packed with dry ice (solidified CO_2) to maintain their frozen condition, and shipped to the collaborator for dissection, cleaning, and preparation of the bone prior to ash analysis. Many of the analysts estimating ash were unfamiliar with dissection technic, while some had had previous experience with the type of manipulation involved. When the dissection was made, it was completed according to the Associate Referee's instructions, the prepared samples preserved in ethanol being used as models.

Then the two series of right tibiae, including those prepared by the collaborator from the frozen specimens and those prepared by the Associate Referee, were crushed, extracted, dried, and ashed according to standard procedure. These data, tabulated and studied by means of statistical methods, are summarized in Table 1. Each analyst obtained approximately the same ash percentage as did the Associate Referee on opposite tibia pairs when both used similar technic. It will be observed that the results on the prepared specimens (Columns 3 and 7) agree very well (refer to Column 11, difference in ash content), the ones obtained by Collaborators* 2, 3, 4, and 8 in respective comparison with those found by the Associate Referee producing exceptionally close agreement, and furthermore this was independent of the nature of calcification or its variability.

In general and excepting those of Nos. 1 and 3, where the agreement was good, collaborators' results with frozen specimens not previously prepared by the Associate Referee for analysis agreed unsatisfactorily with those found by the Associate Referee. The agreement obtained by Nos. 1 and 3 seemingly warrants the belief that the process of freezing does not significantly affect the subsequent estimation of ash. Undoubtedly this point may be determined with increased accuracy if several tibiae, for example the right ones, are frozen, and later the difference in ash content of these and the left tibia pair from the same birds not frozen prior to analysis is observed.

It is clear from Table 1 that neither the nature or extent of calcification of the bones nor the variation in the ash content of the sample employed was of influence when the results obtained were compared. The collaborators usually found a higher average result both with the frozen

* Collaborator 10 used an ashing temperature of 850° C. for 1 hour in place of the usual one of 700° C. for 18 hours.

TABLE 1.—*Statistical summary of comparative procedures for preparation of chick tibiae for ash determination*

COLLABORATOR*	ANALYST	Average ash content and its variability of dried, fat-extracted tibiae (5 or more specimens per group) obtained by— ASSOCIATE REFEREE: PREPARED SPECIMENS (LEFT TIBIAE)		ASSOCIATE REFEREE: SPECIMENS NOT PREVIOUSLY FROZEN (LEFT TIBIAE)		COLLABORATOR: PREPARED SPECIMENS (RIGHT TIBIAE)		COLLABORATOR: FROZEN SPECIMENS (RIGHT TIBIAE)		DIFFERENCE IN ASH CONTENT: COMPARATIVE PREPARED SPECIMENS	DIFFERENCE IN ASH CONTENT: COMPARATIVE FROZEN SPECIMENS	REMARKS ON CALCIFICATION‡
		per cent	*s d*	*per cent*	*s.d*	*per cent*	*s d.*	*per cent*	*s.d.*	*per cent*	*per cent*	
1. E. M. Bailey New Haven, Conn.	W. T. Mathis	46.89	1.34	46.53	0 67	47.70	1.05	46.84†	1.42	−0.81	−0.31	normal
2. H. R. Kraybill Lafayette, Ind.	D. M. Doty	45.14	2 08	44.96	3.43	45 30	2 08	48 26	3.48	−0 16	3.30	normal
3. R. C. Newton Chicago, Ill.	H. E. Robinson	36.90	2.27	36.67	2.46	37.09	2.08	36.57	2.59	−0.19	0.10	subnormal
4. J. H. Mitchell Clemson College, S. C.	D. B. Roderick	45.54	2.14	43.04	2.90	45.40	1.69	45.31†	2.76	0.14	−2.27	normal
5. R. B. Dustman Morgantown, W. Va.	C. E. Weakley, Jr	43.24	3.55	44.00	0.97	44.00	3.62	46.53	1.72	−0.76	−2.53	nearly normal
6. L. E. Bopst College Park, Md.	—	42.98	3 41	41.86	2.64	43 64	2.85	43.74	2 98	−0.68	−1.88	subnormal
7. G. S. Fraps College Station, Tex.	A R. Kemmerer	42.13	4.38	42.70	3 56	42.59	4.42	44.18†	3.69	−0.46	−1.48	subnormal
8. J. J. Taylor Tallahassee, Fla.	C. P. Coleman, E. P. Greene	47.33	0.79	44.26	3 37	47 35	0.81	50.58	3.59	−0.02	−6.32	normal
9. G. H. Marsh Montgomery, Ala.	E. K. Tucker	41.43	2.17	43.02	2.94	41.90	2.05	47.43	1.62	−0.47	−4.41	subnormal
10. W. B. Griem Madison, Wis.	—	47.03	0.79	47.13	1.44	45.60	1.09	45.72	1.56	1.43	1.41	normal

* Given in the order results were received

† Samples partly decomposed when received.

‡ By definition, *Poultry Sci.*, 15, 127 (1936), White Leghorns, four weeks of age, may be characterized as having normal calcification provided 60% of them and the average of the lot are 45% or above in ash content.

and prepared samples, while the variability with the frozen specimen samples but not the prepared ones was with two exceptions greater than was that obtained by the Associate Referee.

To summarize, from these data it appears obvious that concordant ash results may be produced when a standard procedure is employed, provided the bone is previously cleaned and otherwise prepared in the same manner. This conclusion corroborates the data* of previous reports, which suggested that appreciable differences in results may be due to manipulation of the bone by different analysts prior to extraction of crude fat and estimation of ash. Striking evidence supporting this belief is offered by the results of Collaborator 8 (Table 1), who produced comparative ash differences of 31,600 per cent of frozen specimens and those prepared by the Associate Referee.

EFFECT UPON BONE ASH CONTENT WHEN DIFFERENT FAT SOLVENTS WERE USED

In the past the importance of thoroughly extracting lipoid material has been emphasized, because this step is quite essential for accurately estimating ash, part of the source of errors undoubtedly being due to the fact that many different types of solvent (with varying periods of time for extraction) and of apparatus are used. The Associate Referee[1] has demonstrated that the utility of either ethanol or ether as a solvent for extraction is not the same as a combination of the two, comparisons being based upon the solvent property of ethanol followed by ether. Results of statistical study computed by Fisher's method[2] for measuring the significance of difference between paired samples when the distribution of *t* criterion was used, and involving the extraction of the right tibiae either by acetone (dimethyl ketone), isopropyl alcohol (dimethyl carbinol), chloroform (trichloromethane), all of "technical" purity, or by a mixture of half 95 per cent ethanol–half anhydrous ether, the bones otherwise handled like the routine determination of ash, when compared with those that were obtained with the opposite tibia pair (left) extracted by 95 per cent ethanol followed by anhydrous diethyl ether, are given by Table 2. Many birds in a number of groups were used, and the lots displayed different extents of calcification for birds four weeks of age, namely, one lot each of those showing normal, low, and intermediate ash.

When the data are considered separately by solvent used for extraction, the differing results are apparent, those found for tibiae of Lots 1, 2, and 3, extracted by acetone, resembling the former results reported for the solvent ethanol,[3] which showed differences between the use of

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* Some unpublished as yet.
[1] *Loc. cit.*
[2] Statistical Methods for Research Workers, 4th Ed. (1932). Oliver and Boyd, Edinburgh, Scotland
[3] With the exception that the differences between tibia pairs were negative, while these to the contrary are positive.

ethanol compared with the use of ethanol followed by ether were most pronounced when the bones were from birds exhibiting normal calcification. When the bones were poorly calcified the differences were not pronounced or significant by the statistical method of analysis.

TABLE 2.—*Statistical summary of bone ash determinations when the bones were extracted by different solvents*

		AVERAGE ASH CONTENT OF DRIED, FAT EXTRACTED TIBIAE				
LOT NO.	NO. BIRDS	ETHANOL FOLLOWED BY ETHER		DIFFERENCE	STATISTICAL SIGNIFICANCE OF THE DIFFERENCE	REMARKS ON CALCIFICATION
		per cent	*per cent*	*per cent*		
1	17	29.72*	29.36†	0.36	negative	definitely subnormal
2	13	33.61*	33.28†	0.33	negative	definitely subnormal
3	13	46.70*	45.82†	0.88	positive	normal
4	13	30.89*	30.60§	0.29	negative	definitely subnormal
5	12	33.70*	33.23§	0.47	negative	definitely subnormal
6	12	46.75*	45.81§	0.94	positive	normal
7	13	30.64*	28.76**	1.88	positive	definitely subnormal
8	12	38.49*	38.83**	−0.34	negative	subnormal
9	13	46.10*	45.07**	1.03	positive	normal
10	17	31.50*	31.71††	−0.21	negative	definitely subnormal
11	10	37.11*	37.56††	−0.45	negative	subnormal

* Left tibiae were ethanol-extracted for 20 hours in a specially made, intermittent Soxhlet syphoning device followed by 20 hours ether-extraction in a modified Bailey-Walker fat extraction apparatus.

† Right tibiae were acetone-extracted for 72 hours in the Soxhlet apparatus, further extraction producing a negligible amount of crude fat, fresh solvent being used, and the bones turned end for end every 24 hours.

§ Right tibiae were isopropyl alcohol-extracted 120 hours in the Soxhlet apparatus, further extraction producing a small amount of crude fat, fresh solvent being used, and the bones turned end for end every 24 hours.

** Same as Lots 1, 2, and 3, except that the right tibiae were chloroform-extracted.

†† Same as Lots 1, 2, and 3, except that the right tibiae were ethanol-ether mixture-extracted.

Likewise the bones from birds of Lots 4, 5, and 6, extracted with isopropyl alcohol, show differences similar to those extracted by ethanol and acetone. It seems obvious that both acetone and isopropyl alcohol extract the lipoid material of bone less thoroughly than does a combination of ethanol followed by ether. The greater difference in ash content between both Lots 1–3 and 4–6, respectively, by the ethanol-ether method than is evident in the lots when either acetone or isopropyl alcohol was used is an apparent advantage for the ethanol-ether method. A possible disadvantage of acetone is its property of attacking any rubber connections used in the extraction outfit more readily than do isopropyl alcohol and ethanol.

With chloroform as the extracting agent the results are somewhat confusing and contradictory in character. Part of the failure to secure data allowing definite clarity of interpretation may be due to the peculiar corrosive property of chloroform. It attacked nearly everything with which it came in contact, causing rapid corrosion of the metal caps of

the glass vial containers used to preserve the bone samples, and the bones to become friable in character and consequently difficult to crush and extract properly. Furthermore, the amount of solvent adhering to the bone produced an adhesive action on the filter paper during the crushing operation, with the result that it tenaciously resisted attempts at quantitative transference of crushed bone to the ashing dishes after extraction of fat. This difficulty did not occur when other solvents were used.

When a mixture of ethanol and ether was used for extraction, no lot exhibiting a normal ash content was available, and therefore it was impossible to compare the method critically with the ethanol-ether procedure on birds with normal calcification. Thus far the data show that the ash results of bones from poorly and subnormally calcified birds display small differences between the two methods. The mixture of ethanol-ether produces a slightly higher ash content than does ethanol followed by ether, indicating that more lipoid material was extracted with the mixture of solvents (over nearly twice as long a period of time) as with a combination of ethanol followed by ether. It is apparent, however, that the observed differences are not statistically significant and that they are like those shown by the solvent ethanol in a previous report. In practice, when a mixture of solvents was used, the ether passed off through the condenser until a constant evaporating (boiling) mixture of presumably constant physical composition was produced, necessitating the frequent addition of further solvent with the type of apparatus employed until thermal equilibrium in physical composition of the mixture was reached.

There is generally less variability in results shown when the ethanol-ether method was employed than with any other solvent mentioned, the average variability of each based on the corresponding ash contents being as follows:

TYPE OF SOLVENT USED FOR EXTRACTION	AVERAGE VARIABILITY	DIFFERENCE IN VARIABILITY
	per cent	
Ethanol	2.13	
Ethanol followed by ether	2.07	−0.06
Ether	3.18	
Ethanol followed by ether	3.05	−0.13
Acetone	1.5874	
Ethanol followed by ether	1.5870	−0.0004
Isopropyl alcohol	2.46	
Ethanol followed by ether	2.59	0.13
Chloroform	2.47	
Ethanol followed by ether	2.31	−0.16
Mixture of ethanol-ether	3.74	
Ethanol followed by ether	3.47	−0.27

Consequently, less opportunity for introduction of error was presented when the ethanol-ether method was used.

From the evidence presented it seems clear that solvents other than a combination of ethanol followed by ether may be used for extraction, but that for purposes of accuracy and the desirability of reproducing results readily the combination of ethanol followed by ether (owing to the complete extraction of lipoid material of bone, which may be accomplished in a comparatively brief period of time when desired, and to the large mass of data already obtained by this method) appears best suited for the control of bone extraction work.*

COMPARATIVE CRITERIA FOR INTERPRETING RESULTS OF BIOASSAY

When other criteria than ash estimation for interpreting calcification are discussed, it should be recognized that the ash method is probably the most accurate as well as most time-consuming procedure available. Other methods of subjective nature have been criticized because of the possibility of personal bias or faulty judgment of the operator during manipulation. The disadvantage of the time element required for estimating ash is fully offset by the following advantages: Relative inexpensiveness; quantitative character, which makes statistical analysis of the data possible; and adaptability to chemical testing laboratories and to analytical technic. Considerable information may be gained, however, by studying other procedures, possibly not quite so accurate, but several of these have a definite supplementary value, and under certain conditions they may be adapted to replace the ash determination.

From preliminary spade-work done by the Associate Referee during the past two years, the data accumulated in this laboratory indicate the accuracy and the satisfactory utility of several commonly used methods other than the estimation of ash, as (1) diagnosis of bone calcification by Roentgen (X-) rays, a technic for determining the density of calcium salts deposited in bone; (2) mineral analysis (calcium, inorganic phosphorus or both) of blood plasma, a technic for measuring the progress of bone calcification; and (3)[1] the silver nitrate staining method, a technic for visual inspection of the quantity of calcium salts deposited in the bony trabeculae. In most instances the range of the method for interpretation of results may well be an important consideration since the difference in values, as between poorly and normally calcified bones from

* The analyst of collaboration No. 7 suggested that in place of using anhydrous diethyl ether for the latter portion of the extraction dichloroethylene (undoubtedly the symmetrical acetylene dichloride) be used, due to its higher boiling point and increased practicality in climate where the room temperature may be near or above the boiling point of ether.

[1] Results of this study are not reported because the experiments have progressed only to a point where a small number of samples are available for comparison with the ash procedure.

PLATE 1A

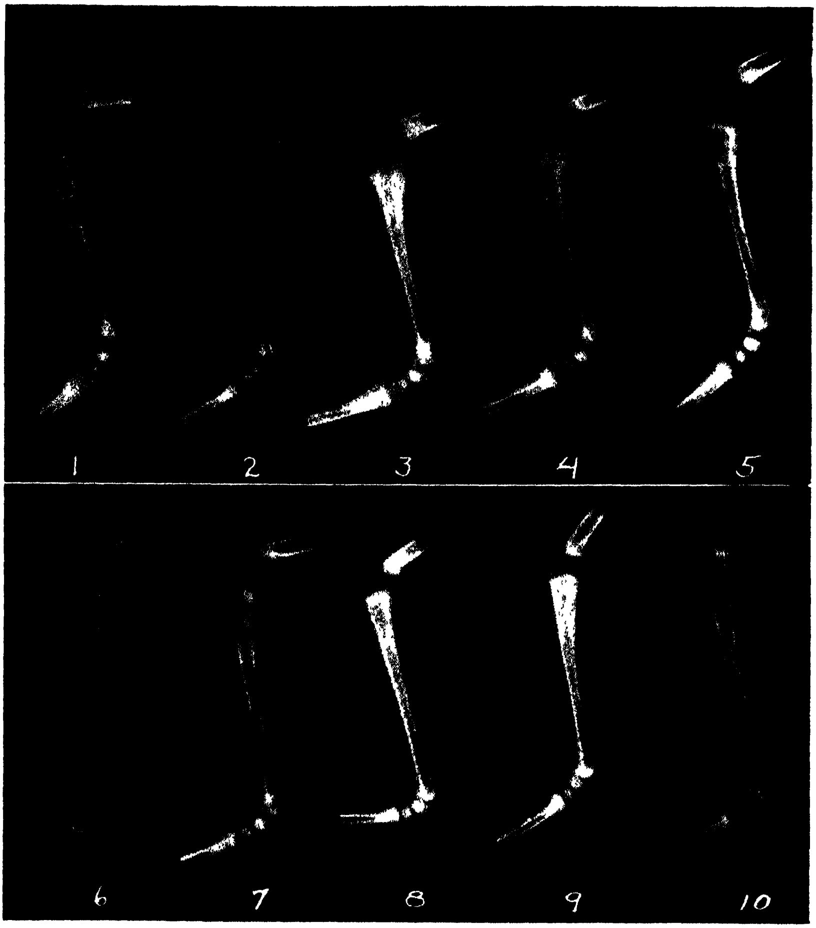

PLATE 1B

chicks eating the A.O.A.C. ration, is used chiefly for expressing or evaluating vitamin D potency of the supplements given. These cases comprise the great majority of those criteria that are used. The methods following are discussed on the basis of this consideration and of probability of error.

METHODS

A beginning was made with several bone samples, when X-ray technic was used and when treated in the following manner: Right legs obtained from White Leghorns four weeks of age were preserved in 50 per cent ethanol prior to their shipment to the laboratory of A. C. Richardson, California Packing Corporation, who kindly exposed them to X-rays and made photographic reproductions which were interpreted visually by means of matching comparisons with a scale reading from 1, very poor calcification, through to 10, normal calcification, the varying extent of this property being shown by the intermediate portions as revealed by the "positives" and "negatives" in Plate 1. At the same time the ash content of the opposite symmetrically-like bone (left tibia) was determined by this laboratory according to standard technic, the results ranging from 26.13 to 47.08 per cent. These estimations of calcification on the left tibia were then compared with the (Richardson) reading of the amount, nature, and density of calcification shown by the opposite bone when it was matched with the standard scale. Results of the association between ash content and X-ray diagnosis (1 to 10) on opposite tibiae based on 70 comparative determinations are shown by a correlation coefficient of 0.954 ± 0.011 (standard error), mean ash content 41.68 per cent ± 0.71 per cent, mean X-ray reading 7.54 ± 0.32, variability of each being shown by a standard deviation of 5.91 and 2.70, respectively, for ash determination and the X-ray technic. The positive correlation between the two methods is thus established as nearly a perfect one, suggesting that a high X-ray reading accompanied a high ash content and a low X-ray reading accompanied a low ash content.

It is interesting to note that the variability or error observed from the corresponding coefficients of variation was higher for the X-ray diagnosis method than when the ash procedure was used, being of the order $2\frac{1}{2}\times$, while the range of the X-ray scale readings was less than half that for the ash determination. The almost perfect correlation existing between the two methods when used by two different technicians unaware of the other's results suggests the use of X-rays as an important supplementary (or replacement) criterion for determining the nature of calcification in the chick provided the cost of apparatus is not prohibitive and expert matching of X-ray photographs with a suitable standard is made. An advantage of the X-ray technic is the fact that the procedure does not

entail destruction of the chick. In this connection it is planned in the future to evaluate critically the error involved in practice when X-ray technic is used.

A similar study was made of calcium and inorganic phosphorus in blood plasma. The method for mineral analysis of chicken blood described by Perish, Lachat and Halvorson[1] was compared with the ash estimation.[2] Occasionally it was necessary to obtain blood from more than one chick, and this caused the pooling of blood samples from several birds near the average in weight of each lot. Results of the association based on 24 comparisons between these blood plasma calcium and inorganic phosphorus determinations and the average ash content of 10 or more birds per lot are presented statistically: correlation coefficient between plasma calcium and ash content, 0.564 ± 0.142; between plasma inorganic phosphorus and ash content, 0.795 ± 0.077; mean ash, 41.56 per cent $\pm$ 1.12 per cent; mean calcium (mg. per 100 cc. of plasma) 12.80 ± 0.28; mean inorganic phosphorus (mg. per 100 cc. of plasma) 6.16 ± 0.22. The variability of each is shown by the standard deviation: 5.47, 1.36, and 1.07, respectively, for ash content, plasma calcium, and inorganic phosphorus. The positive nature of the correlations between comparative methods is thus shown to be high for inorganic phosphorus and ash content and to be statistically significant for calcium and ash content, indicating that the blood minerals in the chick reflect the amount of ash deposited in the bones.

Consideration of the variability or error (coefficient of variation) in these criteria compared with the ash determination suggests that they are of a similar order, being slightly higher for inorganic phosphorus and lower for calcium, the range in values being: ash content, 30.10–46.35 per cent; blood calcium, 9.76–14.68 mg./100 cc. plasma; blood inorganic phosphorus, 3.34–8.06 mg./100 cc. plasma. These figures reveal that the range of ash content is better than $3\times$ that for either plasma calcium or plasma inorganic phosphorus. Owing to the method of selecting pooled-blood specimens the error cannot be ascertained or evaluated with the desired degree of accuracy, and the study of individual variation was impossible. It is planned in the future to continue these experimental studies.

RECOMMENDATION[3]

It is recommended that the study of various factors affecting the estimation of bone ash and of other criteria for interpreting the nature of calcification in young chicks be continued.

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[1] Minnesota Department Agriculture, Dairy & Food, pp. 14–15 (March 1936).

[2] Thanks are due J. W. Perish of this laboratory for assisting analytically in estimating calcium and inorganic phosphorus in chicken blood plasma.

[3] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 49 (1937).

REPORT ON CAROTENE*

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Vitamin A seems to be an essential requirement for live stock. This vitamin may be furnished as such or it may be made in the animal from some provitamin A, such as a carotenoid pigment. Evidence exists that the pigments capable of furnishing vitamin A are alpha, beta, and gamma carotene and kryptoxanthine. Since almost all the vitamin A potency of forage is due to its carotene content a chemical determination of this pigment would be a convenient measure of its potency. Accordingly, the Associate Referee studied this subject with a view to the development of a method for the determination of carotene.

At the time plans were under consideration for collaborative work, the Associate Referee was familiar with the following methods for the estimation of carotene in forage:

H. R. Guilbert, Determination of Carotene as a Means of Estimating the Vitamin A Value of Forage. *Ind. Eng. Chem. Anal. Ed.*, **6**, 452 (1934); W. C. Russell, et al, Colorimetric Determination of Carotene in Plant Tissue. *Plant Physiology*, **10**, 325 (1935); and Method for Determination of Carotene in Farm Feeds. U. S. Dept. Agr. Bur. Dairy Industry.

The Bureau of Dairy Industry method, used in this study, represents the methods in which the carotene isolation is carried out at room temperature. The other method submitted to collaborative study is the Guilbert method as revised by G. S. Fraps. It represents the methods wherein the carotene is isolated after treatment of the sample at the temperature of boiling alcoholic potassium hydroxide.

Two samples, marked No. 1 and No. 2, were submitted to seven collaborators, with the request that carotene be determined by the two following methods and also by any other method familiar to them, about the middle of August. Sample No. 1 was a freshly ground alfalfa leaf meal,[1] and sample No. 2 was a commercial mixed feed plus 10 per cent of alfalfa leaf meal. These samples were well mixed and packed into glass bottles, tightly stoppered, and sealed with paraffin.

REVISED METHOD FOR CAROTENE EXPERIMENT

Alfalfa or Feed.—Reflux 2–6 grams 30 minutes with 40–120 cc. of saturated alcoholic KOH (ethyl alcohol free from aldehydes used). Use care to avoid it, but if any material does collect on the side of the flask, wash it down with alcohol. After refluxing, cool the contents of the flasks, add 50 cc. of ethyl ether, and after shaking for a minute and allowing the sediment to settle, decant the ether alcohol mixture into a 1 liter separatory funnel. Repeat this procedure twice more with 15 cc. portions of ether, then break up the residue by shaking with 5 cc. of 95% ethyl alcohol and 15 cc. of ether. After 2 or 3 additional extractions with ether, the solution usu-

* Carotene in this paper refers to any pigment measured as such in the final petroleum ether solution.
[1] Courtesy of Denver Alfalfa Milling Company

ally comes off colorless, and the residue is discarded. From 125 to 150 cc. of ether is required for the extraction.

Pour gently 100 cc. of distilled water through the alcohol-ether solution in this separatory funnel. Draw off from the bottom of the funnel the alkaline alcohol-water solution containing most of the chlorophylline and flavine flavone, and re-extract by shaking gently 2 times with ethyl ether in another funnel. (If an emulsion is encountered it may be cleared by adding 1 cc. of ethyl alcohol.) Combine the ether extracts and wash with distilled water until free from chlorophylline and alkali. Wash 3 or 4 times by pouring the water through the solution and down the sides of the flask to remove most of the alkali. Remove the remainder by gently shaking the ether solution with 25 cc. portions of water until the wash water no longer gives a color with phenolphthalein.

Transfer the ether solution containing the carotene and xanthophyll to a flask and distil off the ether with diminished pressure.

Dissolve the residue containing the combined carotene and xanthophyll fractions in 30 cc. of petroleum ether, added in three 10 cc. portions and transfer to a small separatory funnel. Extract the xanthophyll from the petroleum ether solution first with 85%, and finally with 90% methyl alcohol (20 cc. portions of alcohol are used).

If in the first extraction with 85% methanol the lower layer is cloudy, draw it off and extract with 10–20 cc. of petroleum ether. Add the petroleum ether extract to the other petroleum ether fraction and continue washing with 85% methanol (5 or 6 extractions with the alcohol and 2 or 3 with 90% methyl alcohol are usually sufficient, but the petroleum ether solution must be washed until the wash alcohol comes through colorless). Finally wash the petroleum ether layer with water, dry over Na_2SO_4 concentrate under vacuum and make up to 10, 25, or 50 cc., depending on the amount of color.

Estimate the amount of carotene in the sample by comparing it colorimetrically against 0.1% potassium bichromate. Put the solution of the sample in the left-hand cup of the colorimeter and set the scale at 0.5 cm., 1 cm., 2 cm., 3 cm., or 4 cm., according to the amount of color present. Vary the depth of the bichromate solution in the right hand cup until the density of color in both cups is equal, and make 8 independent readings, putting them down in milimeters. Average the readings. Make the bichromate readings between 4 mm. and 12 mm. on the colorimeter. If a reading below 4 mm. can not be avoided, make it but repeat the analysis with a larger sample.

By use of the table transform the *millimeter* depth of 0.1% bichromate into p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in the sample by use of the following formula:

$$p = \frac{\text{p.p.m. of carotene (from table)} \times \text{cc. of solution}}{\text{grams of sample} \times \textit{cm.} \text{ depth of sample solution}}.$$

Report carotene to 0.1 p.p.m.

METHOD FOR STANDARDIZING 0.1% POTASSIUM BICHROMATE FOR CAROTENE

Dissolve 1 tube (0.1 gram) of SMA carotene in about 2 cc. of $CHCl_3$ and precipitate with 20 cc. of methanol. Filter, wash with a few drops of methanol, and dry in a desiccator with diminished pressure for about 1 hour. Very carefully weigh out 10 mg. of this purified carotene and dissolve in the smallest possible amount of $CHCl_3$. Dissolve in 100 cc. of petroleum ether. Take 10 cc. and make up to 100 cc. with petroleum ether, which will give a 0.001% carotene solution. Put this solution in the left-hand cup of a colorimeter and set the depth at 5 mm. Vary the depth of

the right-hand cup of the colorimeter, which contains the 0.1% $K_2Cr_2O_7$ to be standardized, until the colors match in intensity. The right-hand side of the colorimeter should read 8.3 mm., but if it doesn't adjust the dichromate solution by adding more $K_2Cr_2O_7$, or more water, until it does read 8.3 mm.

If the carotene solution is set at 4 mm., the $K_2Cr_2O_7$ reading should be 6.5 mm.

Table for calculating carotene

0.1% $K_2Cr_2O_7$	CAROTENE	0.1% $K_2Cr_2O_7$	CAROTENE
mm.	*p.p.m.*	*mm.*	*p.p.m.*
1.0	0.5	6.6	4.1
1.2	0.7	6.8	4.2
1.4	0.8	7.0	4.3
1.6	0.9	7.2	4.5
1.8	1.0	7.4	4.6
2.0	1.2	7.6	4.7
2.2	1.4	7.8	4.8
2.4	1.5	8.0	4.9
2.6	1.6	8.2	5.0
2.8	1.7	8.4	5.2
3.0	1.8	8.6	5.3
3.2	2.0	8.8	5.4
3.4	2.1	9.0	5.6
3.6	2.2	9.2	5.8
3.8	2.3	9.4	5.9
4.0	2.5	9.6	6.0
4.2	2.6	9.8	6.1
4.4	2.7	10.0	6.3
4.6	2.8	10.2	6.5
4.8	2.9	10.4	6.7
5.0	3.1	10.6	6.8
5.2	3.2	10.8	6.9
5.4	3.4	11.0	7.1
5.6	3.5	11.2	7.3
5.8	3.6	11.4	7.4
6.0	3.8	11.6	7.5
6.2	3.9	11.8	7.6
6.4	4.0	12.0	7.8

U. S. BUREAU OF DAIRY INDUSTRY METHOD FOR DETERMINATION OF CAROTENE IN FARM FEEDS

A tentative procedure for the determination of carotene in alfalfa hay adopted in December 1933.

Drill the bales to be analyzed ½ to ¾ of the way through from the alternate corners of one end and grind the drillings to the finest mesh in a Wiley mill. Weigh out the ground samples for carotene (and moisture if desired). (Samples to contain not less than 15 gamma of carotene.) Add about 20 ml. of absolute ethyl alcohol for a 6 gram hay sample (this alcohol may be increased somewhat without difficulty and the sample may be much larger and the alcohol proportion the same). Allow to stand 1 hour to overnight or longer. Then add some low-boiling ligroin, 20 ml. for the 6 gram sample ("F" fraction put out by the Skelly Oil Co., 121 Wacker Drive,

Chicago, Ill., which boils at about 30°–60°). Treat the ligroin several times with H_2SO_4 and with $KMnO_4$ and distil before using. Shake the sample with alcohol and ligroin 5–15 minutes and filter onto a sintered glass funnel, using a little ligroin to wash the hay onto the filter. Return the hay sample to the original container, treat again with ligroin, shake and again filter. Repeat the ligroin extractions 6–10 times. Concentrate the combined filtrate to a small volume in vacuo (75–100 ml.). Treat with 20 ml. of water and shake for 5 minutes to get the alcohol to separate from the ligroin layer. Discard the alcohol-water layer. Saponify the ligroin layer by shaking for 15 min. with 10 ml. of absolute CH_3OH saturated with KOH, adding 25 ml. of 92% CH_3OH, shaking 5 min. more, allowing to stand until the separation is *complete*, and running off the CH_3OH-KOH layer. Repeat this saponification about 4 times. (This removes the chlorophyll and almost all the xanthophyll.) Wash the ligroin solution, containing carotene, by shaking 5 minutes with 92% CH_3OH until the xanthophyll is practically completely recovered from the ligroin. (Frequently only 2 or 3 of these washings are necessary.) Bring the ligroin solution almost to dryness in vacuo, and take up with a higher boiling ligroin ("C" fraction put out by the Skelly Oil Co., which boils at about 90° C.). Wash it with H_2SO_4, treat with $KMnO_4$ and distil before using. Filter the carotene solution in this higher boiling ligroin (No. 42 Whatman paper), bring to volume, and read either on the colorimeter or on the spectrophotometer. (With the colorimeter is used a Hg lamp, a Corning Glass Company filter No. 511, and a $K_2Cr_2O_7$ solution is used as a standard. The match is practically perfect, the filter transmitting only wave lengths of 405 and 436 mμ., and the effect of the former is practically negligible. On the spectrophotometer, the readings are also made with a Hg light at 436 mμ. The correlaton between these methods of reading is very good.)

TABLE 1.—*Results obtained by collaborators* (*only average values given*)

ANALYST	SAMPLE NO. 1			SAMPLE NO. 2		
	DAIRY INDUSTRY PROCEDURE	REVISED GUILBERT PROCEDURE (FRAPS)	REVISED GUILBERT PROCEDURE (PETERSON AND HUGHES)	DAIRY INDUSTRY PROCEDURE	REVISED GUILBERT PROCEDURE (FRAPS)	REVISED GUILBERT PROCEDURE (PETERSON AND HUGHES)
Results expressed in p p m. of carotene obtained by colorimeter, 0.1% $K_2Cr_2O_7$ as standard.						
I	86.6	109.2	95.3	16.4	18.5	16.6
II	97.5	100.0		13.1	15.0	
III	86.4	96.0		13.3	13.5	
IV	74.3	97.5		10.0	12.9	
V	84.4	83.2		15.4	12.0	
VI	88.6	86.9		9.5	12.0	
VII	93.8	91.0		15.9	12.0	
	Max. 97.5	109.2		Max. 16.4	18.5	
	Min. 74.3	83.2		Min. 9.5	12.0	
	Av. 87.4	94.8		Av. 13.4	13.7	
Results by spectrophotometer in p.p.m. of carotene						
I	84.2	98.9		14.0	13.7	
II	89.4	98.0		11.5	14.2	
VI	87.2	87.6		9.6	12.3	
	Av. 86.9	94.8		Av. 11.7	13.4	

In the latter method it was requested that the carotene concentration in solution be measured in the colorimeter as directed under the revised Guilbert method.

COLLABORATORS

The Association appreciates the generous cooperation of the following collaborators in this study:

Lamar Kishlar, V. O. Wodicka and associates, Ralston Purina Co., St. Louis, Mo.
H. R. Guilbert and R. W. Caldwell, University of California, Davis, Calif.
J. S. Hughes, Kansas State College, Manhattan, Kans.
G. S. Fraps and A. R. Kemmerer, Agr. and Mechanical College of Texas, College Station, Texas.
H. R. Kraybill and Mr. Doty, Purdue University Agricultural Experiment Station, Lafayette, Ind.
Oscar I. Struve, Eastern States Cooperative Milling Corp., Buffalo, N. Y.,

COMMENTS OF COLLABORATORS

O. I. Struve.—The Dairy Industry procedure gave emulsions, required too much time, and seemed not to remove all the carotene. The revised method gave results identical with their established procedure in which the saponification is done in KOH saturated methyl alcohol. The analyst did not like the Dairy Industry procedure.

A. R. Kemmerer.—The Dairy Industry procedure is much more tedious and time-consuming than the revised Guilbert method and the Guilbert method is preferred.

H. R. Guilbert.—(This analyst uses peroxide-free ether in the extraction by the revised Guilbert method due to possible destruction of carotene by the peroxides.) The Peterson-Hughes modification of our method in which the saponification mixture is extracted directly with petroleum ether has the advantage of fewer manipulations, there is less danger of destructive impurities in petroleum ether than in ethyl ether, and it is better to work with during hot weather. The disadvantages in our experience are that more care must be exercised to avoid troublesome emulsions, and it is harder to wash the solution free from alkali. The initial extraction is not so clear cut as with ethyl ether as one continues to get small amounts of pigment after many extractions. This we found to be xanthophyll, which is slowly dissolved by petroleum ether. When the petroleum ether comes off only faintly colored, therefore, one can be safe in assuming that all the carotene is removed. The time required is about the same for both procedures.

J. S. Hughes.—In the Dairy Industry procedure the carotene obtained appeared to decrease with the increase in weight of sample taken. Very consistent results were obtained by the Guilbert method when the spectrophotometer was used. We have much more confidence in the latter method than in the Diary Industry procedure. In the Dairy Industry procedure the Skellysolve should be washed with H_2O until free of alkali, as indicated by the phenolphthalien test of the wash water. The presence of traces of alkali and methyl alcohol during the evaporation and subsequent bringing to volume with higher Skellysolve might easily affect the results, especially in spectrophotometric readings.

Mr. Doty.—The technic of the revised method is somewhat simpler and the procedure is more rapid than that of the Dairy Industry method. In the revised method some material that is dissolved by ethyl ether is not dissolved by petroleum ether after the ether solution is evaporated to dryness. This undissolved residue tends to cause the formation of emulsions during the separation of xanthophyll and

carotene, especially with alfalfa meal. The colorimetric results are fairly reliable as indicated by comparison with the spectrophotometer and photoelectric colorimeter. Better results are obtained in the colorimeter by reading with the depth of $K_2Cr_2O_7$ solution between 4.0 and 8.0 mm. instead of 4.0 and 12.0 mm.

V. O. Wodicka.—If the colorimetric method is to be used, it is suggested that another reference solution be substituted for the $K_2Cr_2O_7$. One suggestion along this line is that conversion of dichromate into chromate by means of a little alkali gives a much closer color match. Although this investigator has had no experience with the dye mixture described by Guilbert in his original article, it is thought that such a mixture would make for greater ease of reading.

DISCUSSION OF RESULTS

It may be somewhat of a chance to draw definite conclusions from the data given in the tables, but the general trend can be observed. It might be concluded that the precision of the two methods is about the same with the colorimeter and somewhat better by use of the spectrophotometer. The average values by both the colorimetric and spectrophotometric methods on sample No. 1 showed the revised method to give a higher carotene content. On sample No. 2 the colorimetric method by both procedures gives essentially the same value, while according to spectrophotometric results the revised procedure gives higher values.

The higher results by the revised method may not in all cases mean that the difference is due to more carotene. If, for instance, kryptoxanthine were present it would be present to a greater extent in the carotene fraction in the revised Guilbert method than in the Dairy Industry method and consequently be measured as carotene.

The revised Guilbert method has at least one rather serious objection, namely, extraction with ethyl ether, which later is evaporated off, followed by solution of the residue in petroleum ether. The revised Guilbert method of Peterson and Hughes, which is given below avoids this extraction with ethyl ether but uses instead petroleum ether:

MODIFICATION BY PETERSON AND HUGHES OF GUILBERT METHOD FOR THE DETERMINATION OF CAROTENE IN FORAGE

Weigh out the samples (1–5 grams), transfer to Erlenmeyer flasks, and add 20 cc. of a freshly prepared, saturated solution of KOH in ethyl alcohol to each gram of sample. Fit the flasks with reflux condensers, and boil the contents on a steam bath or hot plate for 30 minutes. If portions of the sample collect on the sides of the flask, wash down with alcohol from a wash bottle. Cool the contents of the flask, add 100 cc. of Skellysolve (b. 60–70°), and after shaking for a minute or so and allowing the sediment to settle, decant the Skellysolve-alcohol mixture into a separatory funnel. Repeat this procedure twice more with smaller quantities of Skellysolve, breaking up the residue in the flask, which sometimes forms an adherent mass, by shaking with a small quantity of 95% alcohol. After two or three additional extractions with Skellysolve, the solution usually comes off colorless and the residue is then discarded.

Pour gently about 100 cc. of distilled water through the alcohol-Skellysolve solution in the separatory funnel. Draw off the alkaline alcohol-water solution from the bottom of the funnel, and re-extract by shaking gently with Skellysolve in another

TABLE 2.—*Complete data submitted by collaborators*
(All results expressed in p.p.m. of carotene)

ANALYST	DAIRY INDUSTRY PROCEDURE			REVISED GUILBERT PROCEDURE (FRAPS)			REVISED GUILBERT PROCEDURE (PETERSON-HUGHES)			REVISED GUILBERT PROCEDURE (FRAPS) EXCEPT EXTRACTED WITH PETROLEUM ETHER ESSENTIALLY PETERSON-HUGHES PROCEDURE
	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER[1]	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	
	gram			*gram*			*gram*			
					Sample No. 1					
I	2	88.04	98.39	3	94.16	115.5	—	—	—	
	2	88.00	97.56							
	5	84.20	88.90	5	100.00	112.8				
	5	86.65	88.90							
	6	77.50	71.38	5	102.67	103.3				
	6	80.50	74.38							
		Av. 84.21	86.58		98.94	109.2	5	94.13	95.33	
II		88.3	97.5		95.0	105.0		98.0		
		Av. 90.5	97.5		101.0	95.0		99.2		
		Av. 89.4	97.5		98.0	100.0		98.6		
				Date analyzed			Date analyzed			
III			76.7	8–20–36		100.0	8–21–36			
			97.5	8–26–36		103.3	8–21–36			
			85.0	8–31–36		90.0	8–25–36			
						89.3	8–31–36			
			Av. 86.4			97.5	9–1–36			
						Av. 96.0				
IV			74.3			97.5				Colorimeter

[1] Obtained by using 0.1% $K_2Cr_2O_7$ as standard according to directions in Guilbert revised procedure.

TABLE 2.—(*Continued*)

ANALYST	DAIRY INDUSTRY PROCEDURE			REVISED GUILBERT PROCEDURE (FRAPS)			REVISED GUILBERT PROCEDURE (PETERSON-HUGHES)			REVISED GUILBERT PROCEDURE (FRAPS) EXCEPT EXTRACTED WITH PETROLEUM ETHER ESSENTIALLY PETERSON-HUGHES PROCEDURE
	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER[1]	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	
	gram			*gram*			*gram*			
V			83.8			83.8				83.8
			85.1			82.5				83.8
			Av. 84.8			Av. 83.2				Av. 83.8
			Dye standard[2]			Dye Standard				Dye standard
			99.2			93.1				93.8
			97.6			92.4				94.4
			Av. 98.4			Av. 92.8				Av. 94.1
VI[3,4]		88.8	91.7		90.0	95.0				
		88.0	90.0		92.5	85.0				
		87.1	85.0		83.0	85.0				
		84.0	87.5		85.1	82.5				
		Av. 87.2	88.6		87.6	86.9				
VII			92.5			90.0				
			95.0			92.0				
			Av. 93.8			Av. 91.0				
Sample No. 2										
I	6	16.20	19.79	5	13.90	21.25				
	6	14.56	17.19	5	13.76	20.60				
	10	12.67	14.35	10	13.42	13.61	10	14.02	16.61	
	10	12.50	14.11		Av. 13.69	18.48				
		Av. 13.98	16.36							
II		12.0	12.7		15.2	16.0		13.2		
		11.0	13.5		13.1	14.0		13.6		
		Av. 11.5	13.1		Av. 14.2	15.0		Av. 13.4		

[2] Obtained by using mixture of naphthol yellow and orange G. The stock dye solution is made by dissolving 3.06 g of naphthol yellow and 0.45 g. of orange G in water and making up to 1 liter. The standard solution is made by diluting 5 cc. of the stock solution to 1 liter and against pure beta carotene, M.P. 182–184° C., is equivalent to 2.4 mg. of carotene per liter. The dyes were obtained from the National Aniline & Chemical Co., N.Y. (orange G Schultz No. 38, naphthol yellow Schultz No. 6).

ANALYST	DAIRY INDUSTRY PROCEDURE			REVISED GUILBERT PROCEDURE (FRAPS)			REVISED GUILBERT PROCEDURE (PETERSON-HUGHES)			REVISED GUILBERT PROCEDURE (FRAPS) EXCEPT EXTRACTED WITH PETROLEUM ETHER ESSENTIALLY PETERSON-HUGHES PROCEDURE
	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER[1]	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	WT. OF SAMPLE	SPECTROPHOTOMETER	COLORIMETER	
	gram			*gram*			*gram*			
				Date analyzed			Date analyzed			
III			13.3	8–20–36		13.9	8–21–36			
			13.3	8–26–36		13.3	8–21–36			
						13.4	8–25–36			
			Av. 13.3			13.5				
IV			10.0			12.9				
V										Colorimeter
			15.5			12.0				12.1
			15.5			12.0				12.1
			15.2			12.0				Av. 12.1
			Av. 15.4			Av. 12.0				
			Dye standard			Dye standard				Dye standard
			17.6			13.6				13.9
			17.8			13.6				13.9
			17.6			13.6				
			Av. 17.7			Av. 13.6				
VI[3]		8.7	8.8		13.2	13.3				
		9.4	9.2		12.4	12.0				
		11.2	10.7		11.8	11.3				
		10.2	9.2		11.8	11.5				
		Av. 9.6	9.5		Av. 12.3	12.0				
VII			15.6			11.7				
			16.2			12.2				
			Av. 15.9			Av. 12.0				

[3] Collaborator VI also reported the following results by the photoelectric colorimeter apparatus described by R. B. Withrow, C. L Shrewsbury, and H. R. Kraybill, *Ind. Eng. Chem. Anal. Ed* 8, 214 (1936)· Sample 1: 80 8, 80 2, 85.8, and 84 8, av 82.9; Sample 2, 8.7, 9.4, 11 7, and 10.5, av. 10.1.

[4] Analyses were made from September 25 to October 23 on samples held in freezing compartment of an electric refrigerator until this date. All other analyses reported were made the latter part of August.

separatory funnel. Combine the Skellysolve extracts and wash with distilled water until free from alkali.

Remove xanthophyll from the Skellysolve solution by extracting first with 85% and finally with 90% methyl alcohol. After 5 or 6 extractions with 85% alcohol, continue washing with 90 per cent MeOH until the solution comes out colorless. Wash the Skellysolve solution containing the carotene twice with distilled water to remove the alcohol and filter into a volumetric flask through filter paper upon which is placed a small amount of anhydrous Na_2SO_4. After making the carotene solution up to volume, determine the concentration by the spectrophotometric method. For each determination make optical density measurements at wave lengths of 4,500, 4,700, and 4,800 Å.U. Using the absorption coefficients calculated for beta carotene at these wave lengths, determine the carotene concentration for each wave length and take the average.

Extinction Coefficients

Wave Length, Å	Skellysolve b. 60–70°	Petroleum ether b. 40–60°
4500	238	243
4550		231
4700	200	207
4800	212	212

Petroleum ether (b. 40–60°) may be substituted for Skellysolve in the above method. The coefficients for petroleum ether were checked by the S.M.A. Corporation.

It will be observed that the original ether extraction has been eliminated entirely, which obviates the necessity of carrying out a single evaporation of solvent during the course of the determination, and excludes the possibility of carotene decomposition, which might occur during the ether evaporation required in the original method. The method is considerably shortened inasmuch as the chlorophyllines, flavones, alkali, and xanthophyll can be removed directly from the Skellysolve.

If a spectrophotometer is not available, the concentration of the carotene in the final solution may be determined by means of a colorimeter. A solution of potassium dichromate containing 0.0173 gram per 100 cc., equivalent to 1 mg. of carotene in 1 liter, may be used as a standard.

It will be noted this method gives essentially the same results as does the Guilbert method revised by Fraps.

RECOMMENDATIONS[1]

It is recommended—

(1) That studies be made to determine the most suitable methods for the extraction and isolation of carotene from feeding stuffs.

(2) That an attempt be made to improve the reference standard used in the colorimetric method.

(3) That studies be made of the use of the photoelectric colorimeter or neutral wedge photometer.

PASTEURIZATION OF DAIRY PRODUCTS

F. W. Gilcreas, State Department of Health, Albany, N. Y., was appointed Associate Referee on Tests for Pasteurization of Dairy Products.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 20, 50 (1937).

CONTRIBUTED PAPERS

EFFECT OF CERTAIN SOLVENTS AND OF SEQUENCE OF EXTRACTION ON THE REMOVAL OF FAT AND ASH CONSTITUENTS FROM THE BONES OF CHICKS*

By R. B. Dustman (Department of Agricultural Chemistry, West Virginia University, Morgantown, W. Va.)

In a recent report by Lachat,[1] the Associate Referee on Technic for Bone Analysis, it is recommended that extraction procedures requiring different lipoid solvents be critically evaluated. Some years ago workers in this laboratory began a study involving analyses of bones from several kinds of experimental animals. At the outset it was recognized that methods of analysis for bones varied widely, not only with respect to sampling but also with respect to procedures for extraction. For this reason some preliminary trials were conducted by the writer to determine the relative effect of certain solvents and also the effect of sequence of extraction, where more than one solvent is used. The trials were made with chicken bones, and therefore are applicable to the problem raised in the report cited.

MATERIALS AND METHODS

The solvents used were ordinary 95 per cent alcohol, absolute alcohol, anhydrous ethyl ether, and acetone (Eimer and Amend B.P. 56–57°, c.p. T.P. Reagent). When absolute alcohol was used the condensers were equipped with calcium chloride tubes as a protection against moisture from the air, but with the other solvents such precaution was not taken.

The bones were from chickens of several ages and planes of nutrition, but were similar in these respects, and hence comparable for the groupings indicated. Whole bones, including the epiphyseal cartilages, were analyzed. For convenience the trials may be divided into two series as described.

Series 1. In the early experiments samples of two similar lots from each chick were made up by combining certain wing and leg bones (ulna, radius, humerus, femur, and tibia) from either side of six-weeks-to-several-months-old chicks. This provided pairs of approximately uniform samples. The paired samples were freed from flesh, crushed between the jaws of pliers, folded in ashless filter paper, and dried in vacuo for 21 hours at 75° C. and at a pressure of approximately 50 mm. of mercury. Since it was desired to calculate all values to a moisture-free basis, no account was taken of moisture content. Subsequent dryings following the various extractions were performed in the same manner. All samples were weighed in glass-stoppered weighing bottles.

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* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 186.

[1] *This Journal*, **19**, 598 (1936).

Extraction in Series 1 was carried out in Soxhlet extractors equipped with cork stoppers covered with metal foil. During extraction the samples were contained in Whatman paper thimbles, from which they were transferred to platinum crucibles for ashing. (In Series 2 the extraction apparatus was considerably improved.) The extraction period was 20 hours for each solvent. Ashing was accomplished in an electric muffle furnace at a temperature not exceeding 600°.

Series 2. Because the paired samples did not permit close comparisons of the solvents used with different individuals it was decided to prepare a large quantity of uniform material, which would permit direct comparisons of various extractive procedures. This was done by cutting air-dry wing and leg bones from several-months-old chicks in a Wiley mill until the material passed through a 1 mm. sieve. This material was then thoroughly mixed, and duplicate samples were chosen for moisture content, the drying being accomplished in vacuo as described under Series 1. Additional losses on continuation of the drying process were determined at intervals up to 176 hours. The Soxhlet extractors used in Series 2 were of Pyrex glass with ground joints, and the paper thimbles used in Series 1 were replaced in Series 2 by Jena porous-bottom, glass extraction shells. The extraction period was 20 hours for each solvent, and the drying period in vacuo after extraction was 21 hours under the same conditions as those described for Series 1.

In Series 2 the extracted samples were not ashed, but instead provision was made for determining the ash content of the extracts removed by the various solvents. The extracts were evaporated to low volume, transferred to porcelain crucibles, and ashed in the same manner as that described for the extracted bones in Series 1.

RESULTS

Series 1. In Series 1 the extractive treatment and sequence of extraction in each instance was as follows:

Paired Sample No.	Solvent and sequence
1a	Ether; 95% alcohol.
1b	95% alcohol; ether.
2a	Ether; absolute alcohol; 95% alcohol.
2b	Absolute alcohol; ether; 95% alcohol.
3a	Absolute alcohol; 95% alcohol.
3b	Ether 90% plus absolute alcohol 10% by volume; Ether 90% plus 95% alcohol 10% by volume.
4a	Ether; absolute alcohol 90% plus acetone 10% by volume; 95% alcohol.
4b	Ether; 95% alcohol 90% plus acetone 10% by volume; 95% alcohol.

TABLE 1.—*Results of analyses of paired chicken bones—various solvents and sequences of extraction used*

Paired sample No.	Original dry sample	Amount soluble in— 1st extraction	2nd extraction	3rd extraction	Total extraction	Ash content, basis of total extraction
	grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1a	6.7202	4.92	3.17	—	8.09	54.88
1b	6.6988	9.08	0.00	—	9.08	54.73
2a	6.5171	7.00	1.58	1.04	9.62	57.95
2b	6.3886	7.63	0.76	1.08	9.47	58.33
3a	6.5609	13.74	1.79	—	15.53	57.49
3b	6.6175	13.76	0.51	—	14.27	57.41
4a	3.7641	11.66	2.11	1.62	15.39	58.28
4b	3.8307	12.77	2.87	0.80	16.44	58.76

Series 2. In Series 2 the extractive treatment and sequence of extraction in each instance was as follows:

Sample No.	Solvent and sequence
1	Ether; absolute alcohol; acetone; 95% alcohol.
2	Ether; acetone; absolute alcohol; 95% alcohol.
3	Absolute alcohol; ether; acetone; 95% alcohol.
4	Absolute alcohol; acetone; ether; 95% alcohol.
5	Acetone; ether; absolute alcohol; 95% alcohol.
6	Acetone; absolute alcohol; ether; 95% alcohol.
7	95% alcohol; ether.
8	95% alcohol; acetone.
9	95% alcohol; absolute alcohol.

TABLE 2.—*Results of analyses of samples of chicken bones—various solvents and sequences of extraction used*

Sample No.	Original dry sample	Amount soluble in— 1st extraction	2nd extraction	3rd extraction	4th extraction
	grams	*per cent*	*per cent*	*per cent*	*per cent*
1	15.0891	14.45	2.30	0.01	0.76
2	14.9510	14.24	0.68	1.57	0.71
3	14.9717	15.66	0.20	0.12	0.82
4	14.9781	15.61	0.07	0.21	0.92
5	14.8928	14.51	0.23	1.33	1.08
6	14.9400	14.68	1.63	0.20	0.86
7	10.2584	16.38	0.03		
8	10.3769	16.33	0.07		
9	10.2689	16.15	0.25		

Table 3 shows the ash content of the extracts listed in Table 2.

TABLE 3.—*Ash content of extracts from various solvents and sequences of extraction*

SAMPLE NO.	1ST EXTRACTION	2ND EXTRACTION	3RD EXTRACTION	4TH EXTRACTION
	per cent	*per cent*	*per cent*	*per cent*
1	1.08	13.53	18.75	18.87
2	1.13	4.97	13.03	21.27
3	2.08	11.04	8.07	17.77
4	lost	5.46	4.09	22.52
5	0.92	5.66	12.89	33.83
6	0.91	18.95	0.00	38.27
7	2.21	11.11		
8	2.29	10.97		
9	2.36	16.13		

Table 4 shows the loss on drying the original material used in Series 2, the drying being accomplished in vacuo for 21 hours at 75° C. and at a pressure of approximately 50 mm. of mercury. Additional losses on continuation of the drying process are shown at intervals up to 176 hours.

TABLE 4.—*Losses on drying samples of chicken bones in vacuo for various periods*

SAMPLE	ORIGINAL SAMPLE	LOSS AFTER 21 HOURS	ADDITIONAL LOSS DURING SUCCESSIVE DRYING PERIODS				
			21–42 HRS.	42–70 HRS.	70–92 HRS.	92–112 HRS.	112–176 HRS.
	grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	3.1130	6.21	0.28	0.23	0.13	0.00*	0.05
B	3.2980	6.21	0.27	0.23	0.13	0.00*	0.06

* Slight gains due possibly to atmospheric conditions at time of weighing.

DISCUSSION

Although rather unsatisfactory on the whole because of sample variability, nevertheless the results recorded in Table 1 indicate certain differences in action for the solvents compared. From Samples 1a and 1b, ether used prior to 95 per cent alcohol removed 61 per cent of the total extractives, while in the reverse order of extraction ether failed to remove additional extractives. From Samples 2a and 2b the 95 per cent alcohol removed approximately equal and not inconsiderable amounts after ether and absolute alcohol had first been used. A similar result is shown for Sample 3a, where 95 per cent alcohol follows absolute alcohol. These data suggest that at least some of the material removed by 95 per cent alcohol is not soluble in either ether or absolute alcohol. The results obtained with mixed solvents for Samples 4a and 4b also show a greater solvent power for 95 per cent alcohol than for ether or absolute alcohol.

For reasons previously stated a second series of experiments was planned in order to allow for a closer control of sample material and also for an examination of the ash content of the various extracts. A comparison of the results of the first extractions (Table 2) shows that ether and acetone removed approximately equal amounts of extractive and that

these amounts were smaller than those removed by absolute alcohol and by 95 per cent alcohol. This is as one might anticipate. The mean values are 14.35, 14.60, 15.64 and 16.29 per cent, respectively, for ether, acetone, absolute alcohol, and 95 per cent alcohol.

The results of the sequence of extraction studies shown by the second, third, and fourth extractions in Table 2 are interesting. Ether following 95 per cent alcohol (No. 7) removed only a slight amount of extractive. This outcome is in close agreement with that shown in Table 1, Sample 1b. Somewhat larger and almost equivalent quantities were removed by ether following absolute alcohol and acetone (Nos. 3 and 5). Acetone following ether removed appreciably more than when following absolute alcohol or 95 per cent alcohol (Nos. 2, 4, and 8). Absolute alcohol following ether removed a large amount of extractive, but lesser amounts following acetone and 95 per cent alcohol (Nos. 1, 6, and 9).

In the column of third extractions it may be seen that acetone (Nos. 1 and 3) removed the least amounts of material, followed in order by ether (Nos. 4 and 6) and by absolute alcohol (Nos. 2 and 5), which removed the largest amounts by a considerable margin.

The fourth extraction in each case was accomplished with 95 per cent alcohol, with the results shown in the corresponding column of Table 2. In each instance the amount removed was considerable.

By the end of the second extraction period some samples had been subjected to the action of the same solvents, the only difference being the sequence of treatment. It is interesting, therefore, to compare the total extractives at this point and again after the third and fourth extractions. Table 5 shows these totals, together with those for Samples 7, 8, and 9.

TABLE 5.—*Total extract at end of second, third, and fourth extraction periods*

SAMPLE NO.	TOTAL EXTRACT AT END OF—		
	2ND EXTRACTION	3RD EXTRACTION	4TH EXTRACTION
	per cent	*per cent*	*per cent*
1	16.75	16.76	17.52
2	14.92	16.49	17.20
3	15.86	15.98	16.80
4	15.68	15.89	16.81
5	14.74	16.07	17.15
6	16.31	16.51	17.37
7	16.41		
8	16.40		
9	16.40		

At the end of the second extraction period ether-acetone gave a total of 14.92 per cent as compared with 14.74 per cent for acetone-ether. The results are so nearly identical that the difference due to sequence is probably not significant. Where absolute alcohol was one of the solvents the

outcome was quite different. Ether-absolute alcohol yielded a total of 16.75 per cent against 15.86 per cent for absolute alcohol-ether. Also, acetone-absolute alcohol yielded a total of 16.31 per cent against 15.68 per cent for the reverse sequence. These last two differences, too large to be attributed to experimental error, suggest that where absolute alcohol precedes ether and acetone some influence is operating to render the total extract smaller in amount than where absolute alcohol follows these solvents. This effect is not entirely obliterated by the third and fourth extraction periods notwithstanding the fact that 95 per cent alcohol was the fourth solvent in each case.

The ash content of the material removed by the various solvents is also interesting, and particularly so for the first extraction in each case. In Table 3 it may be noted that the extract removed by acetone contained less ash than that removed by any of the other three solvents. Ether removed appreciably more ash than did acetone, and each of the two alcohols removed more than twice as much ash as did acetone. Calculating the average ash content of the first extract in each case to grams of ash removed from 100 grams of original dry bone, one arrives at the following values: 0.1335, 0.1584, 0.3252, and 0.3725 for acetone, ether, absolute alcohol, and 95 per cent alcohol, respectively. In comparing ash values beyond the first extraction it should be remembered that some of these values were determined on relatively small amounts of material and involved transfer of the extract to crucibles for ashing. For these reasons their accuracy beyond the first extraction is not comparable. Nevertheless, certain tendencies may be clearly traced. The ash content for the absolute alcohol extracts from Samples 1, 2, 5, 6, and 9, for example, are 13.53, 13.03, 12.89, 18.95, and 16.13 per cent, respectively. In like manner the extractives removed by 95 per cent alcohol in the fourth extraction carry considerable and fairly constant quantities of ash.

Table 4 shows the losses resulting from continued drying of the material over a long period. The values indicate that the material used was reasonably uniform in moisture and volatile materials.

SUMMARY

A study was made of the relative effect of acetone, ether, absolute alcohol, and 95 per cent alcohol on the removal of fatty material and ash from the bones of chickens. The solvents were used singly in various sequences. The results obtained were as follows:

(1) Acetone and ether removed approximately equivalent quantities of extractive.

(2) The ash content of the acetone extract was lower than that of the ether extract.

(3) Absolute alcohol and 95 per cent alcohol removed considerably more extract and much more ash than did either acetone or ether.

(4) When absolute alcohol preceded acetone and ether as the first solvent the total extract was somewhat less than when the sequence was reversed.

(5) Ether or acetone used after 95 per cent alcohol removed only very small quantities of extractives, but 95 per cent alcohol used after ether or acetone removed much additional material.

DETECTION OF DECOMPOSITION PRODUCTS IN BUTTER AND CREAM*

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INTRODUCTION

In general practice, cream for butter-making is separated on the farm, although in certain progressive areas whole milk is received and separated at the creameries. Farms supplying cream for butter-making purposes are frequently small, and the cream is often allowed to accumulate with little or no cooling. It may be exposed to prevailing temperatures for several days, or even weeks. Commercial cream always contains lactic acid bacteria. As produced on the ordinary farm there are nearly always present other types of organisms, which manifest themselves after a more or less lengthy incubation period. At the beginning there is a rapid increase in acidity, due principally to the action of lactic acid bacteria, and the characteristics acquired are those of clean sour cream which, of course, cannot be considered as decomposed. There can be no objection to the manufacture of butter from clean cream that has undergone merely the normal lactic acid souring. However, in many cases lactic fermentation is accompanied by biochemical changes in the various cream constituents that give rise to abnormal substances, rendering the cream decomposed, as evidenced by organoleptic characteristics well known in the industry under such terms as putrid, cheesy, yeasty, bitter, moldy, fruity, et cetera.

The investigation described was designed to discover if there might be decomposition products or indices of decomposition in the butter made from such unfit cream. It was also desired to study certain chemical and physical characteristics of cream that might be used commercially to determine its fitness for butter making.

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† The Food Division of the Food and Drug Administration contributed many valuable suggestions. L. H. Chernoff suggested the basic method for indole P A. Clifford suggested the use of the photometer in the determination of indole. C S Myers assisted in the development of the distillation method. W. R. North participated in the bacteriological phases of the work.

It seems reasonable to believe that when butter is churned from decomposed cream some of the products of decomposition are retained in the butter and not entirely lost in the buttermilk. However, preliminary experiments indicated that these products did not affect the ordinary butter fat constants, with the exception of the acidity of fat. As will be later developed, acidity of fat proved to be a useful index.

It is generally known that indole is a product of decomposition of proteins containing tryptophane, such as casein. It is slightly soluble in an aqueous medium and is very soluble in the ordinary fat solvents. It seemed reasonable to believe, therefore, that if indole is formed in cream, a material portion of it would occur in the butter made therefrom. This proved to be the case, and the quantities of indole found in butter made from decomposed cream were often many times those found in butter made from fresh cream.

Wildman[1] has shown the usefulness of mold as an index of decomposition in cream and butter made therefrom.

A modified Kreis test, a determination of peroxides, and a study of the time of filtration in the Greene method[2] were found to be of limited value.

Studies were also made on objective indices of decomposition in cream itself to supplement organoleptic tests commonly used in the creamery. Acidity, hydrogen-ion concentration, formol titration, and mold were studied and found to have diagnostic value.

METHODS OF ANALYSIS

The following methods for butter and cream were used. Some are well-known methods slightly modified; others are new methods from which published or unpublished available material was utilized.

I.—BUTTER

(A).—PREPARATION OF SAMPLE

Place butter in a closed glass container in an oven maintained at 50° C. and allow to stand for approximately 14 hours (required to yield a clear filtrate). Filter through a folded filter paper (C. S. & S. 1117½). If the fat contains any water or is not perfectly clear, filter it a second time.

(B).—ACIDITY OF FAT

REAGENTS

(a) *Benzene.*—Use the best available quality of benzene. If it is not neutral, titrate 15 cc. with the 0.05 N sodium ethylate (b) and correct subsequent results accordingly.

(b) *Sodium ethylate.*—0.05 N. Dissolve a piece of metallic Na approximately 1 cc. in volume in 800 cc. of absolute alcohol. Titrate 10 cc. of 0.1 N HCl with this solution and add the calculated volume of absolute alcohol to make the solution 0.05 N. Standardize each time used.

[1] *This Journal*, 20, 93 (1937).
[2] *Food Industries*, 7, 442 (1935).

DETERMINATION

Accurately weigh approximately 5 grams of the filtered fat into an Erlenmeyer flask and dissolve in approximately 15 cc. of benzene; add 5 drops of phenolphthalein indicator (1+100) and titrate with 0.05 N sodium ethylate. (The end point is reached when the yellow color changes to orange.) Report results as cc. of 0.1 N sodium ethylate per 100 grams of fat.

(C).—DISTILLATION METHOD FOR INDOLE

REAGENTS

(Applicable in the presence of the various artificial butter flavors and commercially used butter colors.)

(a) *Chloroform.*—U.S.P.

(b) *Phosphoric acid.*—85%, C.P.

(c) *Phosphoric-aldehyde.*—Dissolve 0.4 gram of paradimethylaminobenzaldehyde in 5 cc. of acetic acid and mix with 92 cc. of H_3PO_4 and 3 cc. of concentrated HCl. Purify the paradimethylaminobenzaldehyde as follows: Place 100 grams in a 250 cc. Claissen distilling flask, inserting the stem of the flask into an ordinary 250 cc. distilling flask until the end of the stem is in the middle of the receiving bulb. Attach the side-arm tube of the receiver to a source of vacuum. Play a jet of cold water upon the receiving bulb to condense the distillate. Use synthetic rubber* stoppers throughout. Distil under a vacuum of less than 16 mm. of mercury and collect only the constant boiling portion, discarding the first and last parts of the distillate. Dissolve the solid distillate in a minimum of alcohol. Dilute with a tenfold amount of distilled water. Filter off the precipitate, wash with water, and dry over H_2SO_4 in a vacuum desiccator. The yield should be approximately 85% with good commercial paradimethylaminobenzaldehyde.

(d) *Acetic acid-Ether.*—50–50 by volume of acetic acid and U.S.P. ether. Apply the U.S.P. XI peroxide test to ether, and if positive, obtain purer ether. Purify the acetic acid by the following method: To 500 cc. of acetic acid in an *all glass* still, add an excess of $KMnO_4$ (about 25 g.) and 20 cc. of H_2SO_4. Allow to stand 2 hours and distil. (A suitable trap should be used as a still head, and glass beads may be used to prevent bumping.)

(e) *Standard indole.*—Weigh accurately 20 mg. of indole into a 200 cc. volumetric flask and dilute to mark with 95% alcohol (Solution A). Pipet 2 cc. of Solution A into a 200 cc. volumetric flask and dilute to mark with 95% alcohol (Solution B). Solution B should be freshly prepared whenever used; Solution A may be used for 10 days if preserved in the ice box. Solution B contains 1 gamma (0.001 mg.) of indole per 1 cc.

(f) *Alcohol.*—95%. Check the specific gravity, and have the alcoholic content within the range of 94–98% (this is critical).

(g) *Hydrochloric acid.*—Dilute 5 cc. of concentrated HCl to 100 cc. with water.

DISTILLATION APPARATUS

Use a separate steam generator for each unit. For the distillation flask use a 500 cc. round-bottomed Kjeldahl connected to a straight bore condenser (water-jacket 450 mm. in length) through a Reichert-Meissel bulb (*Methods of Analysis, A.O.A.C.*, 1935, 414). Use synthetic rubber stoppers and allow a minimum exposure to the rubber tubing at the connection between the steam generator and distillation flash. Protect the receiving flask from heat with an asbestos board.

DETERMINATION

Have all glassware scrupulously clean and, with the exception of the 125 cc.

* "Duprene" used in this work.

separatory funnel and 50 cc. cylinder, rinse it with alcohol just before use. (The separatory funnel and cylinder must be completely dry when used.)

Measure 100 cc. of melted butter fat at approximately 40° C., into a 250 cc. glass-stoppered measuring cylinder, and fill to the 250 cc. mark with the 95% alcohol. Shake vigorously for 2 minutes. (If the fat should solidify place the cylinder in a 60° C. water bath for a few minutes, as the butter fat layer must be liquid after the two layers separate.) Let stand until the two layers are approximately separated and shake for an additional 2 minutes. Allow to stand until the alcohol and butterfat layers are completely separated.

Pipet 125 cc. of the alcohol layer into a 500 cc. Kjeldahl flask and connect to the steam distillation apparatus described previously. (The 125 cc. aliquot portion contains one-half the indole in the butterfat.) Apply steam and distil to 450 cc., conducting the distillation at such a rate that it is completed in about 90 minutes. Maintain the volume of the solution in the steam distillation flask at approximately 70 cc. by the application of heat. Use 600 cc. of distilled water in the steam generator and discard the residue after each determination. Transfer the distillate to a separatory funnel. Add 5 cc. of the dilute HCl. Wash the condenser and receiving flask with 25 cc. of $CHCl_3$ and add the $CHCl_3$ to the separatory funnel, shaking vigorously for at least 1 minute. Allow the layers to separate and draw off the $CHCl_3$ into a second separatory funnel containing 400 cc. of distilled water. Repeat the extraction with 20 cc., and then with 15 cc. of $CHCl_3$. Combine the first two $CHCl_3$ extracts and shake vigorously for about 15 seconds to remove excess alcohol. Draw off the $CHCl_3$ through a pledget of cotton inserted in a filter funnel into a 125 cc. separatory funnel (must be dry). Add the third $CHCl_3$ extract to the wash water from the first two, shake vigorously for 1 minute and filter into the same 125 cc. separatory funnel.

To the $CHCl_3$ extract, add 10 cc. of the phosphoric-aldehyde reagent and shake vigorously exactly 2 minutes at a frequency of approximately 200 per minute. Allow to stand exactly 10 minutes and separate the acid layer as completely as possible into a glass-stoppered 50 cc. cylinder (must be dry). Let the separatory funnel stand another 2 minutes and again drain into the cylinder. Repeat this procedure until a further 2 minutes' standing gives no additional acid. (Extreme care must be exercised to avoid any $CHCl_3$ in the acid layer, and no attempt should be made to drain the acid from the bore of the stopcock.) Dilute the mixture in the cylinder to approximately 40 cc. with the acetic-ether reagent, mix, and cool. Fill with the acetic-ether mixture to the 50 cc. mark and mix. If not brilliantly clear, filter the solution through a small paper, keeping the funnel covered with a watch-glass to prevent evaporation. Transfer the solution to a suitable photometer cell and determine the scale reading. Reading should be made within 15 minutes. If the color is too deep to read in the smallest cell available, make appropriate dilutions with the acetic-ether mixture. Subtract from the scale reading the blank as determined below with the same still. Convert the corrected scale reading to gamma of indole by referring to the standard curve described below. Report indole as gamma per 50 cc. of fat.

DETERMINATION OF BLANKS

Determine the blank on each distillation apparatus, using 125 cc. of alcohol and continuing as outlined above, beginning "connect to steam distillation," in the third paragraph under "Determination." Record the average of 5 photometer readings. Blank readings should be obtained on all cells.

PREPARATION OF STANDARD CURVE

Pipet the required amount of standard indole (Solution B) into the distillation flask to make a series of standards of 1, 2, 5, 7, 10, 15, 20, and 30 gamma. Add

sufficient alcohol to make 125 cc. Continue as directed under "Determination of Blanks." Take the readings in cells of approximately 25, 50, and 100 mm. lengths and plot a separate curve for each cell after subtracting the blank reading for the still in which the determination is made. The photometer should be fitted with a light filter that transmits within a narrow wave length band (560 millimicrons. See K. S. Gibson, *J. Optical Society*, **25**, 131 (1935), E & A. Cat. (1936), No. 20658/6, p. 228) corresponding to near the maximum absorption band for the indole color.

(D).—ALCOHOL DILUTION METHOD FOR INDOLE

(Results slightly higher than those by the distillation method. Method can be used in the absence of yellow A B or O B with little sacrifice of accuracy.)

Follow the distillation method to the end of the second paragraph under "Determination." Pipet 125 cc. of the alcohol layer into a separatory funnel, add 325 cc. of distilled water, 5 cc. of the dilute HCl, and 25 cc. of $CHCl_3$. Complete the determination as directed in the distillation method, beginning "Shaking vigorously for at least 1 minute," in the third paragraph under "Determination."

(E).—EXTRACTION METHOD FOR INDOLE

(Rapid method requiring a minimum of manipulation, but not applicable to butter colored with yellow A B or yellow O B, widely used butter colors. Strict adherence to detail is absolutely necessary. Its greatest usefulness is in tracing the development of indole in butter made from cream under variable conditions and as a sorting-out test in the routine examination of samples of uncolored butter. The apparent indole by this method is slightly higher than the indole obtained by the distillation method.)

REAGENTS

Same as described for the distillation method, except that $CHCl_3$ is used for dilution of the standard indole instead of 95% alcohol.

DETERMINATION

Measure 50 cc. of filtered butterfat into a *dry* 250 cc. pear-shaped separatory funnel with a 50 cc. graduated cylinder. Start the analysis within a few hours after the butterfat is filtered, because with some samples there is a tendency to low results if the filtered fat stands for a day or more. Wash the butterfat remaining in the graduate into the separatory with 50 cc. of $CHCl_3$. Add 15 cc. of the phosphoric-aldehyde reagent and shake vigorously exactly 2 minutes at a frequency of about 200 per minute. Allow to stand exactly 10 minutes and separate the acid layer as completely as possible into a *dry* glass-stoppered 100 cc. cylinder. Let the separatory funnel stand another 2 minutes and again drain into the cylinder. Repeat until a further 2 minutes' standing gives no additional acid. Use extreme care to avoid any $CHCl_3$ in the acid layer, and do not attempt to drain the acid from the bore of the stopcock.

Dilute the mixture in the cylinder to about 80 cc. with the acetic-ether reagent, mix, and cool. Fill with the acetic-ether mixture to the 100 cc. mark and mix. If not brilliantly clear, filter through a small paper, keeping the funnel covered with a watch-glass to prevent evaporation. Transfer the solution to a suitable photometer cell and determine the scale reading. Reading should be made within 15 minutes. If the color is too deep to read in the shortest cell available, make appropriate dilution with the acetic-ether mixture. Subtract the blank described under "Preparation of Standard Curve" from the scale reading, and convert the corrected scale reading to gamma of indole by referring to the standard curve described below. Report indole as gamma per 50 cc. of fat.

PREPARATION OF STANDARD CURVE

Prepare a series of standards containing 0, 1, 2, 5, 7, 10, 15, 20, 30, and 50 gamma of indole, each dissolved in 100 cc. of $CHCl_3$ in a 250 cc. separatory funnel. Treat each standard identically as directed in the preceding method, beginning, "Add 15 cc. of the phosphoric-aldehyde, etc." in the first paragraph under "Determination." Record the average of 5 readings for each standard on suitable coordinate paper, after subtraction of the 0 or blank reading. Take readings in cells of approximately 25, 50, and 100 mm. lengths and prepare a separate curve for each cell. The photometer should be fitted with the light filter described in the distillation method.

(F).—MOLD COUNT

Use the method reported by Wildman in *This Journal*, 20, 93 (1937).

II.—CREAM

(G).—ACIDITY

REAGENTS

(a) *Sodium hydroxide*.—0.1 *N*.

(b) *Phenolphthalein*.—1 gram per 100 cc. of alcohol.

DETERMINATION

Thoroughly mix and weigh 18 grams of cream into an Erlenmeyer flask. (With some creams of high fat content, particularly if badly decomposed, it is necessary to mix in the most intimate fashion in order to obtain a representative sample.) Add 20 cc. of distilled water and 0.5 cc. of the phenolphthalein solution. Titrate with 0.1 *N* NaOH with continuous agitation until there appears a faintly pink color that does not fade after 1 minute. During the titration hold the flask against a white background in order to reveal more clearly the first appearance of a pink color. Report results as per cent lactic acid (1 cc. 0.1 *N* NaOH = 0.0090 gram) on the fat-free basis.

(H).—FORMOL TITRATION

REAGENT

(a) *Formol solution*.—To 50 cc. of 40% formaldehyde solution add 0.5 cc. of phenolphthalein and neutralize with 0.1 *N* NaOH.

DETERMINATION

To the neutralized solution from Method G, add 2 cc. of freshly prepared formol solution and continue the titration with 0.1 *N* NaOH to a permanent, faintly pink color. This second titer, expressed as cc. of 0.1 *N* NaOH per 100 grams of fat-free cream, represents the formol titration.

(I).—HYDROGEN-ION CONCENTRATION

DETERMINATION

Determine *p*H electrometrically, using a quinhydrone (gold) electrode. After mixing the cream and quinhydrone, allow the mixture to come to equilibrium before taking the reading. Record the temperature at the same time.

(J).—MOLD IN CREAM

Use the method reported by Wildman in *This Journal*, 20, 93 (1937).

DISCUSSION OF METHODS OF ANALYSIS

Acidity of Fat (*Method B*).—Based on the A.O.A.C. method for acidity of ether extract of eggs. Only the detail of manipulation is varied. On

two collaborative samples examined in Chicago and St. Louis by five analysts the following results were obtained:

Analyst	*Acidity of Fat (cc. 0.1 N)* *Sample I*	*Sample II*
I.S., St. Louis	6	30
S.A., St. Louis	7	31
J.C., Chicago	5	29
K.M., Chicago	5	30
R.V., Chicago	5	30
Average	5.6	30.0

Distillation Method for Indole (Method C).—Based on the separation of the indole from the butterfat by extraction with 95 per cent alcohol, steam distillation of the alcohol extract, extraction of the distillate with chloroform, and the determination of the indole in the chloroform extract. The well-known reaction of paradimethylaminobenzaldehyde with indole, as modified by L. H. Chernoff (unpublished data), is used.

Attempts at complete extraction of indole with successive portions of alcohol resulted in such a volume of alcohol as to make impossible the final determination of indole. In studying the partition of indole between butterfat and alcohol it was found that the indole is distributed in direct proportion to the relative volumes of the two immiscible liquids. The method finally adopted makes only one extraction and the volume of alcohol and butterfat is so adjusted that an aliquot part of the alcohol yields one-half the indole in the sample.

The following experiments illustrate the rather remarkable distribution of indole between butterfat and alcohol:

(a) 50 mg. of indole was shaken with 100 cc. of butter fat and 150 cc. of alcohol as directed in Method C. Exactly equal portions (slightly more than 2 cc.) of the alcohol and the fat were accurately measured, and each was diluted to 100 cc. with $CHCl_3$. Two cc. of each solution was then diluted to 100 cc. with $CHCl_3$ and the indole was determined by Method E. The following results were obtained:

Sample No.	*Indole (gamma) found in equal aliquot parts of—* *Fat phase*	*Alcohol phase*
KA523	8.48	8.60
KA609	8.40	8.68

(b) 100 cc. of a sample of fat from a badly decomposed cream, containing a large amount of indole, was made to 250 cc. with alcohol, the mixture was shaken as directed in Method C, and indole was determined by Method D in 125 cc. of the alcohol layer. The residual 125 cc. of fat plus alcohol was again made to 250 cc. with alcohol, 125 cc. was taken, and indole was determined as before. This process was repeated 5 times and approximately 12 cc. of fat was extracted in the 6 fractions. The results follow:

Alcohol fraction	*Indole (gamma) in 125 cc.*	*Per cent of previous fraction*
1	648	—
2	324	50
3	160	49
4	74	46
5	35	47
6	17	49

(c) Indole was determined by the distillation method on 25 samples of commercial butter. At the same time, twice the amount of indole recorded below in the third column was added to a second 100 cc. portion of fat from each of these same samples, and the distillation method again was applied. In the second column below is recorded the amount of indole found in 125 cc. of the alcoholic extract of the original fat; in the fourth column is recorded the amounts found in 125 cc. of the alcoholic extract of the duplicate samples containing the added indole. As may be noted, in each case, within limits of experimental error, one-half of the indole added to the 100 cc. fat sample was recovered in the 125 cc. of extract. Therefore, the indole found in 125 cc. of the alcoholic extract represents the total indole present in 50 cc. of butterfat.

SAMPLE NO.	ORIGINAL INDOLE FOUND	INDOLE ADDED TO FAT	RECOVERY (TOTAL INDOLE)	
	gamma per 50 cc. fat	*gamma per 50 cc. fat*	*gamma per 50 cc. fat*	*per cent*
IA404	5.8	10	15.9	101
IA402	7.6	10	17.5	99
IA405	5.0	10	14.8	98
IA406	4.6	10	13.3	87
IA407	4.5	10	14.5	100
IA408	4.2	10	13.7	95
IA409	2.2	10	12.3	101
KA500	1.9	10	11.6	97
KA502	2.2	10	10.8	86
KA504	1.7	10	11.4	97
IA410	6.1	10	16.1	100
IA412	8.5	10	19.1	106
IA506	2.4	40	40.2	95
IA501	4.3	40	41.0	92
IA517	2.6	40	41.6	98
IA516	1.4	10	11.4	100
IA518	2 8	10	12.4	96
IA511	2.2	10	11.9	97
IA514	2.7	10	13.0	103
IA515	2.0	10	11.5	95
IA519	2.5	10	12.0	95
IA508	1.9	10	10.9	90
IA509	1.4	10	11.2	98
IA513	2.0	10	12.1	101
IA510	2.0	10	11.6	96

Separate subdivisions of two samples of butterfat were examined by 5 analysts in two different laboratories with the following results:

Analyst	*Indole (gamma per 50 cc. fat)* *Sample I*	*Sample II*
S.A., St. Louis	4.2	6.2
	4.3	6.4
J.H.C., Chicago	3.9	6.8
	4.1	6.6
K.L.M., Chicago	4.3	6.0
	4.3	7.0
I.S., St. Louis	4.0	6.4
	4.6	6.6
R.V., Chicago	4.2	6.4
	4.2	6.3
	4.21	6.47

To test the validity of the distillation procedure as a measure of true indole, colors developed from pure indole solutions were compared on a photoelectric spectrophotometer with those developed from a series of commercial butter samples. Both the pure indole solutions and the butter samples were carried through the entire distillation procedure (Method C) with the slight variation noted below.

The curves in Plate I were obtained by photoelectric spectrophotometric determinations on the developed indole color. Typical curves on two samples of commercial butter containing 23 gamma and 8.4 gamma of indole per 50 cc. of fat are shown. A curve is also shown giving the average values for pure indole (four determinations involving different amounts of indole). For convenience in comparison, all absorption coefficients were recalculated by multiplying each ordinate value by the ratio of the absorptions at 5700 Ångström units.

Acetic acid was used to develop the final color for both the standards and the samples. (High room temperatures prohibited use of the usual acetic-ether solution.) The readings of the developed color required approximately 1¾ hours, during which time slightly more than 5 per cent fading occurred. The readings were obtained in the same way for each sample, beginning with the 5700 Ångström unit wave length and then taking alternate readings on each side of this setting.

Inspection of the butter curves shows that they differ somewhat from the pure indole curve. Since it is well known that skatole often occurs with indole in the tryptophane breakdown and since skatole distils and reacts with *p*-dimethylaminobenzaldehyde to form a color which absorbs more strongly in the "red" than does indole, it is probable that much of the slight deviation of the absorption curves for butter from that of pure indole is due to skatole.

Alcohol-Dilution Method for Indole (*Method D*).—Follows the distillation method except that the alcoholic extract is diluted with distilled water instead of being distilled. Table 7 shows 33 samples of butter on which indole was determined by the extraction method and checked by the alcohol-dilution method, and 42 other samples by the extraction and distillation methods. Comparison of the results shows that when referred to the extraction method, the alcohol-dilution method gives results only slightly higher than the distillation method.

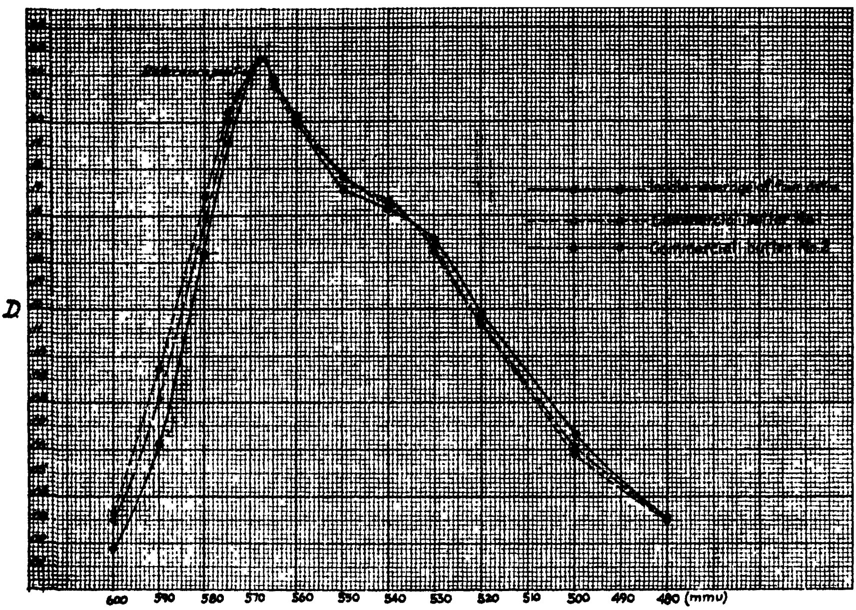

PLATE I

ABSORPTION CURVE FOR PURE INDOLE AND FOR INDOLE ISOLATED FROM COMMERCIAL BUTTERS—BY DISTILLATION METHOD

Extraction Method for Indole (*Method E*).—Indole is determined without previous separation from the butterfat by treatment of a chloroform solution with a phosphoric acid solution of paradimethylaminobenzaldehyde.

On account of the large amount of butterfat present, side reactions, which increase the apparent amount of indole, are to be expected. The method is somewhat empirical and it is necessary to define closely the conditions under which the determination is made. With respect to the various conditions the following observations have been made:

(1) The amount of apparent indole increases somewhat as the amount of Reagent (c) is increased.

(2) As the amount of the sample is increased there is a small decrease in the amount of apparent indole per unit volume.

(3) The amount of hydrochloric acid in the phosphoric-aldehyde reagent exercises a profound effect on the result. In the preparation of standards the amount of HCl is unimportant, but with butterfat maximum photometer readings are obtained in the presence of 3 cc. of HCl per 100 cc. of reagent.

(4) The presence of as much as 4% of added water in the phosphoric-aldehyde reagent does not decrease recoveries from either chloroform or butterfat.

(5) Alcohol up to 2% in the chloroform causes no change in the apparent recovery. Therefore, U.S.P. chloroform, which contains about 1% alcohol, is satisfactory.

(6) Acetic acid in the reagent up to 16% does not affect the result. Approximately 5% of the acetic acid is used simply to facilitate the solution of the paradimethylaminobenzaldehyde.

(7) When the concentration of paradimethylaminobenzaldehyde is increased in the reagent there is an apparent increase in the amount of indole.

The following table shows recovery experiments on 26 samples of commercial butter:

SAMPLE NUMBER	ORIGINAL INDOLE FOUND	INDOLE ADDED	RECOVERY (TOTAL INDOLE)	
	gamma per 50 cc fat	*gamma per 50 cc. fat*	*gamma per 50 cc. fat*	*per cent*
IA404	7.9	10.0	18.4	105
IA402	7.9	10.0	15.3	74
IA405	6.4	10.0	16.4	100
IA406	5.8	10.0	15.8	100
IA407	6.6	10.0	15.6	90
IA408	4.6	10.0	14.4	98
IA409	4.2	10.0	13.4	92
K500	3.5	10.0	12.4	89
K502	3.4	10.0	12.0	86
K504	3.4	10.0	11.6	82
IA410	7.8	10.0	16.8	90
IA411	7.2	10.0	16.0	88
IA412	10.1	10.0	18.8	87
K501	5.5	40.0	42.0	91
K506	3.6	40.0	38.2	87
K517	4.6	40.0	41.0	91
K516	3.6	10.0	13.4	98
K513	3.3	10.0	12.0	87
K518	4.8	10.0	15.1	103
K509	2.3	10.0	11.6	93
K510	4.9	10.0	12.8	79
K503	2.9	10.0	11.8	89
K511	3.6	10.0	12.0	84
K519	4.4	10.0	14.7	103
K514	5.1	10.0	12.9	78
K515	4.5	10.0	12.6	81

Two samples of butter were examined by four chemists in Chicago and two chemists in St. Louis with the following results:

Analyst	*Indole (gamma per 50 cc. fat)* *Sample I*	*Sample II*
R.V., Chicago	10.3	5.5
J.H.C., Chicago	9.7	4.9
K.M., Chicago	9.8	5.3
J.O.C., Chicago	9.4	5.0
I.S., St. Louis	9.4	5.6
S.A., St. Louis	8.9	4.8
Average	9.58	5.18

Acidity of Cream (Method G).—Widely used in the industry. In the literature the deterimination of acidity of cream usually directs the analyst to "titrate with 0.1 *N* alkali, using phenolphthalein indicator." A similar method (tentative) for milk is given in *Methods of Analysis, A.O.A.C.*, 1935, p. 264. Results are reported on the fat-free basis, since the fat of cream for butter making varies widely (20–60 per cent) and does not contribute significant quantities of acid. While this method of reporting is unusual in the literature, the results are of more value than when no correction is applied for fat content. Fat determinations were made by the Babcock method.

Formol Titration (Method H).—A simplification of the Sörenson method for amino nitrogen in meat products (*Methods of Analysis, A.O.A.C.*, 1935, p. 364). It is useful for certain field work where only a minimum of equipment is available.

Hydrogen-ion Concentration (Method I).—Widely employed in determining *p*H in cream and milk.[1]

EXPERIMENTAL PART

The following experiments were made to test the diagnostic value of the foregoing methods in the detection of decomposition. These experiments fall naturally into studies on cream and butter churned therefrom in the laboratory, and on commercial butter.

I. Cream Experiments

Effect of Pure Lactic Acid Cultures (Table 1)

Three lots of commercial cream of the best obtainable quality were used. The cream was separated at the milk station, subjected to partial pasteurization by flashing at 66°C. for 1 to 2 minutes, cooled to 7°C., and brought to the laboratory under refrigeration within 24 hours after milking. Each lot was thoroughly mixed and divided into a number of approximately 3 liter portions. Each portion, except controls D2i, Ei and Fa, was sterilized at 120°C. for 30 minutes. Controls D2b, D2c, Eb, Ec, and Fd were treated like the other portions, except that no culture was added.

[1] Horrall, Purdue Univ. Agr. Exp.Sta. Bull. No. 401 (1935); Parks and Barnes, *Ind. Eng. Chem.. Anal. Ed.*, 7, 71–72 (1935).

Each portion, except as noted above, was inoculated with pure lactic acid cultures, obtained from the U. S. Bureau of Dairy of Industry, and allowed to stand at room temperature for a varying number of days. At the end of the storage period the acidity of the cream was determined. It was then churned into butter, commercial practices being followed as closely as possible. That is, it was neutralized to 0.25 per cent acidity with sodium carbonate, pasteurized at 68°C. for 25 minutes, cooled, and allowed to ripen overnight after the addition of a pure lactic culture. Indole, acidity of fat, and mold were immediately determined on the resultant butter. Aside from a slight burnt flavor the butter in every case was normal. Analysis showed it to contain 80–83 per cent fat.

TABLE 1.—*Action of pure lactic cultures on sterilized cream*

SAMPLE NUMBER	CREAM			BUTTER		
	CULTURE	STORAGE AT 20–30°C.	ACIDITY (LACTIC)	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
		days	*per cent*	*cc 0.1 N*	*gamma per 50 cc. fat*	*per cent*
Series D						
D2i(a)	none	0	0.17	11	1	0
D2b	none	0	0.17	11	2	0
D2c	none	30	0.17	11	2	0
D2d	GK	3	1.24	13	2	0
D2e	GK	6	1.52	12	2	0
D2g	GK	22	1.54	14	2	0
D2h	GK	30	1.57	14	2	0
Series E						
Ei(a)	none	0	0.17	11	Lost	0
Eb	none	0	0.17	13	2	0
Ec	none	32	0.24	11	1	0
Ee	B2	4	2.14	13	2	0
Eh	B2	32	2.95	15	1	0
Series F						
Fa(a)	none	0	0.19	14	2	0
Fd	none	32	0.25	14	2	0
Fe	GK	5	1.36	17	2	0
Ff	GK	8	1.64	17	2	0
Fg	GK	14	1.76	16	2	0
Fh	GK	34	1.67	18	2	0
Fi	39aH	16	3.03	17	2	0
Fj	39aH	34	3.19	20	2	0
Fk	39a	16	1.84	17	2	0
Fl	39a	34	1.97	18	2	0
Fm	GK+B2	16	2.82	17	2	0
Fn	GK+B2	34	2.79	19	2	0
Fo	GK+39a	16	1.85	17	2	0
Fp	GK+39a	34	1.78	17	2	0

(a) Not sterilized.

There was no significant increase in acidity of fat, indole, or mold when sterile cream was subjected to normal lactic acid fermentation, even when high lactic acid developed in the cream. When no culture was added there was no significant change in the acidity of the cream or in the acidity of fat, indole, or mold, even over protracted periods. The inoculated cream showed in every case a normal development in acidity.

While there is no significant increase in the acidity of the fat, it is interesting to observe a slight increase in those subdivisions showing a high lactic acid development (Table 1).

Effect of Inoculation with Decomposed Cream (Table 2)

This experiment was conducted in a manner similar to the previous experiment, except that minute amounts of organoleptically decomposed cream were used to inoculate the sterile cream. In the column headed "Type of cream used for inoculation" is given the organoleptic characteristics of the cream used for inoculation. The inoculating material was obtained from current receipts at a creamery, and the organoleptic characteristics were pronounced. The 16 day samples showed degrees of organoleptic decomposition beyond that usually noted in market cream. While it was impossible to churn the 32 day samples, the results on the acidity and indole of the extracted fat may be accepted as valid.

It will be noted that indole in the fat develops very slowly (see particularly Samples J3 and J4). As extreme decomposition develops the acidity of both the fat and the cream may decrease.

This experiment indicates that the organisms present in decomposed cream, when inoculated into sterile cream, will produce in the fat churned therefrom high acidity and mold counts. They may also produce high

TABLE 2.—*Sterilized cream inoculated with decomposed cream*

SAMPLE NUMBER	CREAM			BUTTER		
	TYPE OF CREAM USED FOR INOCULATION	STORAGE AT 20–30°C.	ACIDITY (LACTIC)	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
		days	*per cent*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
J1	none	0	0.20	14	2	0
J7	none	32	0.29	15	2	0
J2a	rancid	16	2.38	202	4	100
J2b(a)	rancid	32	1.76	152	3	—
J3a	metallic	16	2.53	184	9	100
J3b(a)	metallic	32	3.34	290	74	—
J4a	putrid	16	2.36	114	9	100
J4b(a)	putrid	32	2.00	444	2300	—
J5a	cheesy	16	2.21	53	3	98
J5b(a)	cheesy	32	1.65	132	17	—
J6a	yeasty	16	2.41	175	5	100
J6b(a)	yeasty	32	2.30	90	3	—

(a) Impossible to churn. Determinations made on dried petroleum ether extract.

indole. By inference it indicates that when cream decomposes the butter churned therefrom may show high indole, acidity of fat, or mold counts.

Rate of Decomposition of Commercial Cream (Table 3)

Seven samples of cream separated on the farm, each representing a single milking, were collected from farms that were operated under generally good sanitary conditions. Each sample was iced down immediately after separation and shipped to the laboratory. On receipt it was subdivided into portions of about 2 liters each. Each portion was placed in a clean, sterile, 1 gallon aluminum covered milk pail with a tightly fitting cover. It was then stored at the temperature and for the length of time shown. The storage time includes 2 days in transit at temperatures of 7–13°C. The temperature given for the 2 day storage period is that of the cream when received in the laboratory. At the end of the storage period acidity, pH, and formol titration were taken on each can of cream. It was then churned into butter, as in previous experiments, and acidity of fat, indole, and mold counts were determined on each batch of the resulting butter.

As a general rule there was observed a progressive increase in all the indices of decomposition as the cream acquired age, the rate of increase being more rapid the higher the temperature. It is of interest to note below the cream acidities at which, under the various storage conditions, definite organoleptic decomposition was evident.

Series No.	*Storage Temperature* °*C.*	*Storage Period* *days*	*Acidity* *per cent*
IB7	22	18	1.49
IB5	22	15	1.63
IB6	22	13	1.35
IB4	25	11	1.57
IB2	25	8	2.02
IB1	35	7	2.32

There is a general belief among creamery operators that the acidity of cream reaches a maximum and then decreases on further storage. Only series IB6 exhibited this phenomenon and then only after a storage period of 30 days, long after organoleptic decomposition had begun.

Particularly noteworthy is series IB3, which developed acidity of approximately 1 per cent in 9 days at 12°C. and showed no further significant increase through the 23rd day. However, another portion of this same cream, which was held at 12°C. for 23 days, warmed to 25°C., and held thus for an additional 15 days, developed 3.21 per cent acidity.

The results for pH and formol titration values require no discussion. The acidity of fat shows a general tendency to increase materially on storage. Series IB7 and IB6 show some tendency to fluctuate. Series IB4 and IB1 reach a comparatively high value, falling off only after prolonged storage.

Indole shows a material increase in value in the various series, illustrated as follows:

Sample	*Temperature °C.*	*Indole shows marked increase—*
IB3	12	None* in 23 days
IB7	22	Between 22nd and 29th day
IB5	22	Between 15th and 36th day
IB6	22	Between 17th and 24th day
IB4	25	Between 15th and 21st day
IB2	25	Between 8th and 11th day
IB1	35	Between 7th and 10th day

* Another portion of this same cream was held at 12°C. for 23 days, warmed to 25°C., and held for an additional 15 days. It developed 157 gamma of indole per 50 cc. of fat.

In each series organoleptic decomposition appeared before there was any marked increase in indole.

TABLE 3.—*Rate of decomposition of commercial cream*

				CREAM			BUTTER		
	SAMPLE NUMBER*	STORAGE	TEMP. STORAGE	ACIDITY (LACTIC)	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
		days	*°C.*	*per cent*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
	Series IB3								
Fat 43%	IB3a	2	13	0.32	5.75	13	7	4	0
	IB3b	9	12	0.95	4.42	18	11	3	41
	IB3c	16	12	1.02	4.44	18	21	4	77
	IB3d	23	12	1.03	4.30	19	24	4	73
	IB3e(a)	38(b)	25	3.21	3.63	44	89	157	89
	Series IB7								
Fat 28%	IB7a	2	7	0.19	6.23	16	19	5	0
	IB7b	6	22	1.11	4.37	29	60	5	39
	IB7c	10	22	1.26	4.26	25	32	4	38
	IB7d	14	22	1.47	4.11	26	80	5	92
	IB7e	18(b)	22	1.49	—	32	123	9	88
	IB7f	22(b)	22	1.47	4.07	42	52	9	98
	IB7g	29(b)	22	1.93	3.87	33	86	18	95
	Series IB5								
Fat 36%	IB5a	2	10	0.20	6.51	15	19	4	0
	IB5b	4	22	0.99	4.51	26	20	6	0
	IB5c	6	22	1.28	4.36	25	23	10	5
	IB5d	8	22	1.48	4.20	26	30	9	27
	IB5e	11	22	1.40	4.10	34	36	9	22
	IB5f	15(b)	22	1.63	4.00	29	58	9	67
	IB5g	36(b)	22	2.37	3.76	34	102	37	56

* The arabic numeral designates the cream lot.
(a) On 23rd day temperature raised to 25° C., which was maintained until 38th day.
(b) Definitely decomposed—organoleptic observation.

TABLE 3.—*Continued*

	SAMPLE NUMBER*	STORAGE	TEMP. STORAGE	CREAM: ACIDITY (LACTIC)	CREAM: pH	CREAM: FORMOL TITRATION	BUTTER: ACIDITY FAT	BUTTER: INDOLE (EXTRACTION METHOD)	BUTTER: MOLD
		days	*°C.*	*per cent*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
	Series IB6								
Fat 31%	IB6a	2	10	0.19	6.41	12	17	2	0
Fat 31%	IB6b	4	22	0.93	4.47	22	21	4	0
Fat 31%	IB6c	7	22	1.11	4.23	26	37	5	56
Fat 31%	IB6d	10	22	1.30	4.02	28	99	5	71
Fat 31%	IB6e	13(b)	22	1.35	4.00	25	95	5	78
Fat 31%	IB6f	15(b)	22	1.48	3.92	25	36	8	79
Fat 31%	IB6g	17(b)	22	1.63	3.90	29	53	13	99
Fat 31%	IB6h	24(b)	22	1.79	3.82	38	74	92	96
Fat 31%	IB6i	30(b)	22	1.99	3.80	38	55	188	90
Fat 31%	IB6j	36(b)	22	1.85	3.78	33	60	51	86
	Series IB4								
Fat 35%	IB4a	2	7	0.17	6.52	15	10	5	0
Fat 35%	IB4b	4	25	1.00	4.44	31	11	6	2
Fat 35%	IB4c	7	25	1.67	3.93	30	56	5	3
Fat 35%	IB4d	11(b)	25	1.57	3.78	31	80	6	20
Fat 35%	IB4e	15(b)	25	2.78	3.60	43	101	8	11
Fat 35%	IB4f	21(b)	25	3.08	3.54	39	100	24	31
Fat 35%	IB4g	28(b)	25	3.30	3.54	35	85	11	2
	Series IB2								
Fat 45%	IB2a	2	10	0.20	6.13	15	8	4	0
Fat 45%	IB2b	3	25	0.84	4.52	23	12	4	0
Fat 45%	IB2c	5	25	1.20	4.30	24	41	5	40
Fat 45%	IB2d	8(b)	25	2.02	3.81	26	60	7	75
Fat 45%	IB2e	11(b)	25	2.56	3.61	29	72	35	88
Fat 45%	IB2f	15(b)	25	3.30	—	36	72	170	83
Fat 45%	IB2g	21(b)	25	3.34	—	41	87	280	73
	Series IB1								
Fat 36%	IB1a	2	10	0.34	5.46	15	19	4	0
Fat 36%	IB1b	3	35	0.98	4.53	18	37	4	1
Fat 36%	IB1c	4	35	1.39	4.12	21	50	4	20
Fat 36%	IB1d	5	35	1.73	3.86	26	66	3	15
Fat 36%	IB1e	6	35	2.20	3.78	31	69	4	13
Fat 36%	IB1f	7(b)	35	2.32	3.70	30	85	7	34
Fat 36%	IB1g	10(b)	35	2.86	3.61	34	75	62	1

Composition of Commercial Cream (Tables 4, 5, and 6)

In Tables 4, 5, and 6 are recorded the analyses of a large number of samples of commercial cream for butter making, and of butter made therefrom. Aside from Table 4, which contains a few samples of cream separated at the creamery and a few samples taken from the farm, all samples were from commercial shipments of farm-separated cream taken at the factory. Each sample was either iced and shipped to the laboratory or (if nearby) brought to the laboratory immediately after the cream was collected. All these samples were collected during July, August, and September, 1936.

From the odor and taste, the conditions at the creamery, and the condition of delivery, the samples in Table 4 were judged to be cream of good commercial quality.

The cream reported in Table 5 is classified as "questionable." Such cream is rejected by organoleptic test by some operators, and accepted by others as low quality, second-grade cream.

Table 6 gives data on cream that is classed organoleptically as decomposed. That is, decomposition had reached the stage where its condition was plainly evident from the sense of taste and smell. None of the samples in Tables 4, 5, and 6 had a "weedy" flavor.

Acidity of cream, mold in cream, hydrogen-ion concentration, and formol titrations were obtained on the samples immediately after receipt in the laboratory. The cream was then churned into butter, as in previous experiments, and the acidity of fat, indole, and mold counts were determined on the resultant butter. Indole was determined by the extraction method. Samples are arranged in each table in order of ascending acidity of cream.

The average results from Tables 4, 5, and 6 show the following interesting relationship with the degree of observed decomposition:

	CREAM			BUTTER		
	ACIDITY (LACTIC)	MOLD	FORMOL	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*	*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
4 (65 good)	0.47	0	17	15	4	0
5 (53 questionable)	2.07	2.1	31	21	6	30
6 (60 decomposed)	2.36	3.1	34	35	30	47

As was to be expected in retrograde biochemical changes of so complex a nature, not all the organoleptically decomposed cream in Table 6 yielded butter having high values for acidity of fat, indole, or mold.

TABLE 4.—*65 Samples of cream of good quality*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA409	.12	0	6.65	15	4	4	0
IA407	.15	0	6.37	13	5	7	0
IA411	.15	0	6.49	16	5	7	0
IA408	.16	0	6.49	15	5	5	0
IA317	.16	0	6.36	15	16	2	0
IA315	.16	0	6.41	13	11	2	0
IA316	.17	0	6.43	14	17	2	0
IA320	.17	0	6.46	14	28	3	0
IA311	.18	0	6.43	14	—	—	—
IA305	.18	0	6.29	15	31	4	0
IA318	.19	0	6.37	15	16	4	0
IA304	.21	0	6.09	16	—	—	—
IA410	.21	0	6.43	17	8	8	0
IA412	.22	0	6.30	16	11	10	0
IA302	.22	0	6.05	16	14	3	0
IA404	.22	0	6.31	16	6	8	0
IA401	.23	0	6.15	12	19	10	0
IA331	.26	0	5.73	16	17	3	0
IA330	.28	0	5.42	13	11	3	0
IA314	.28	0	5.98	15	19	5	0
IA402	.29	0	5.90	14	7	8	0
IA301	.30	0	6.04	16	8	3	0
IA308	.31	0	5.91	17	7	2	0
IA336	.31	0	5.98	11	50	3	0
IA333	.34	0	5.50	16	6	3	0
IA307	.37	0	5.74	17	13	6	0
IA332	.38	0	5.33	11	16	4	0
IA403	.38	0	5.55	14	11	9	0
IA405	.41	0	5.00	16	11	6	0
IA313	.44	0	4.68	18	13	4	0
IA328	.46	0	5.08	18	12	3	0
IA335	.46	0	5.26	14	11	10	0
IA406	.47	0	5.00	16	12	6	0
IA334	.49	0	5.16	15	14	3	0
IA214	.51	0	4.80	13	20	11	0
IA329	.51	0	5.18	15	20	10	0
IA303	.52	0	4.95	17	7	3	0
IA319	.54	0	4.68	14	17	2	0
IA323	.55	0	5.54	14	6	3	0
IA213	.57	0	4.98	18	9	7	0
IA312	.62	0	4.99	18	14	3	0
IA306	.63	0	4.94	17	18	3	0
IA310	.63	0	4.69	16	14	3	0

TABLE 4.—*Continued*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA325	.63	0	5.03	16	14	3	0
IA326	.64	0	4.65	15	14	3	0
IA203	.66	0	4.57	16	19	2	1
IA202	.67	0	4.55	17	17	3	0
IA321	.67	0	4.87	24	10	3	0
IA327	.68	0	4.80	21	37	3	0
IA201	.69	0	4.57	17	19	3	0
IA204	.69	0	4.46	21	10	2	0
IA309	.69	0	5.01	20	11	3	0
IA208	.71	0	4.72	25	11	4	0
IA212	.71	0	4.69	21	16	4	0
IA207	.72	0	4.71	25	16	3	0
IA210	.72	0	4.65	27	16	3	0
IA205	.75	0	4.54	21	13	4	0
IA215	.75	0	4.60	23	14	3	0
IA322	.76	0	5.02	17	8	4	0
IA324	.76	0	4.70	17	8	2	0
IA211	.77	0	4.55	19	46	3	0
IA216	.79	0	4.73	18	17	3	0
IA206	.85	0	4.77	27	13	2	0
IA217	.86	0	4.55	18	14	4	0
IA209	.99	0	4.65	31	14	4	0
Average	.47	0	—	17	15	4	0
Maximum	.99	0	6.65	31	50	11	1
Minimum	.12	0	4.46	11	4	2	0

TABLE 5.—*53 Samples of questionable cream*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA117	.79	0.0	4.55	19	8	2	1
IA122	.89	0.0	4.33	21	11	26	0
IA120	.90	0.0	4.56	19	—	—	—
IA116	.95	5.2	4.40	30	14	9	77
IA118	1.00	1.5	4.45	26	7	4	0
IA124	1.02	0.0	4.43	26	15	4	0
IA125	1.04	0.0	4.30	25	21	5	3
IA123	1.10	0.0	4.16	24	14	3	0
IA119	1.15	0.0	4.31	23	22	4	39
IA141	1.24	4.0	3.89	21	16	4	55

TABLE 5.—*Continued*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA137	1.26	3.7	3.94	25	17	5	9
IA126	1.37	2.0	4.00	27	18	7	19
IA121	1.39	1.0	4.14	28	23	6	22
IA136	1.42	6.4	3.88	37	28	4	66
IA127	1.45	1.5	3.87	29	—	—	9
IA128	1.46	0.0	3.92	29	22	4	9
IA132	1.52	1.9	3.84	20	17	7	46
IA143	1.67	0.0	3.57	22	20	7	39
IA130	1.71	0.0	3.84	29	12	10	2
IA112	1.86	3.6	3.71	32	42	6	56
IA101	1.93	0.0	3.77	25	19	4	0
IA138	1.96	1.8	3.69	27	17	7	26
IA150	2.08	7.8	3.69	34	29	9	93
IA134	2.10	0.0	3.59	30	17	2	10
IA114	2.13	4.2	3.73	27	19	8	13
IA113	2.14	2.0	3.52	28	52	7	36
IA129	2.14	2.6	3.63	34	31	4	16
IA153	2.17	3.2	3.80	27	18	3	67
IA108	2.24	0.0	3.53	27	8	2	0
IA111	2.25	2.5	3.57	29	19	4	2
IA133	2.28	3.6	3.71	42	30	4	41
IA139	2.28	0.0	3.74	30	12	5	0
IA131	2.29	5.4	3.62	37	29	10	14
IA104	2.34	2.0	—	38	17	3	55
IA107	2.37	7.8	3.51	31	15	7	78
IA135	2.44	0.0	3.64	32	13	4	0
IA148	2.45	0.0	—	34	9	11	0
IA102	2.48	5.7	3.53	31	28	4	89
IA144	2.56	2.8	3.51	26	17	10	100
IA105	2.60	0.0	—	33	25	6	0
IA147	2.68	0.0	3.53	33	18	7	2
IA145	2.70	0.0	3.59	35	21	10	0
IA109	2.76	4.0	3.54	30	18	6	87
IA115	2.79	3.0	3.60	36	44	4	42
IA140	2.83	1.5	3.63	37	11	8	0
IA110	2.87	0.0	3.62	29	52	3	32
IA149	2.98	5.0	3.61	39	16	5	77
IA142	3.03	0.0	3.65	48	28	4	2
IA103	3.07	0.0	3.53	35	17	3	0
IA146	3.12	3.0	3.55	40	29	4	58
IA152	3.22	7.6	3.52	39	16	6	90
IA106	3.44	0.0	—	46	16	4	1
IA151	3.62	4.5	3.58	46	12	6	81
Average	2.07	2.1	—	31	21	6	30
Maximum	3.62	7.8	4.56	48	52	26	100
Minimum	.79	0.0	3.51	19	7	2	0

TABLE 6.—*60 Samples of decomposed cream*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLS (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA20	1.15	0.2	4.20	24	11	4	10
IA17	1.22	0.0	4.73	21	38	12	0
IA 5	1.27	0.2	4.57	24	29	11	20
IA18	1.41	0.2	3.54	24	17	3	9
IA16	1.52	0.2	4.65	20	30	3	7
IA21	1.59	0.2	3.90	20	32	8	39
IA14	1.60	2.4	3.87	30	44	6	41
IA28	1.69	3.0	3.79	32	36	26	86
IA41	1.80	0.1	3.77	26	17	7	0
IA 1	1.89	2.4	3.61	50	39	4	55
IA24	1.92	4.0	3.93	—	16	87	37
IA50	2.02	3.9	3.62	24	14	9	62
IA58	2.04	3.7	—	27	36	10	23
IA19	2.05	1.4	3.67	17	18	3	31
IA45	2.05	4.5	3.64	27	61	4	47
IA43	2.08	2.3	3.60	25	23	79	54
IA47	2.08	4.9	3.64	29	42	5	46
IA10	2.12	0.0	3.57	30	18	22	32
IA 8	2.13	4.8	3.44	31	31	24	88
IA56	2.13	5.0	—	42	49	2	0
IA25	2.18	2.6	3.83	33	32	61	—
IA30	2.18	9.7	—	37	32	45	100
IA12	2.21	7.2	3.59	37	39	30	100
IA 6	2.22	1.0	3.84	33	59	11	62
IA39	2.22	2.0	—	30	18	5	25
IA 3	2.28	3.0	3.48	29	22	13	80
IA32	2.29	3.9	3.72	36	65	7	66
IA26	2.32	0.0	3.57	30	27	9	—
IA 2	2.34	7.9	3.72	54	30	7	62
IA54	2.36	0.9	3.46	31	28	6	2
IA53	2.42	0.0	3.43	25	23	5	7
IA46	2.44	0.1	3.58	24	17	8	0
IA34	2.45	0.2	3.52	35	16	6	31
IA49	2.45	2.3	3.66	23	18	7	38
IA57	2.46	3.8	—	31	58	4	17
IA 9	2.49	3.1	3.57	38	34	10	87
IA35	2.51	6.9	3.68	44	40	58	91
IA51	2.52	6.5	—	29	21	6	100
IA44	2.53	0.2	—	25	15	6	20
IA36	2.59	0.2	3.53	33	42	5	31
IA13	2.60	2.8	3.87	51	72	5	85
IA15	2.65	2.7	3.44	22	23	7	70
IA22	2.66	8.1	3.64	44	97	755	100
IA 7	2.68	2.8	3.46	24	17	7	65

TABLE 6.—*Continued*

SAMPLE NUMBER	CREAM				BUTTER		
	ACIDITY (LACTIC)	MOLD	pH	FORMOL TITRATION	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
	per cent	*mm.*		*cc. 0.1 N*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
IA29	2.73	0.0	3.56	35	10	5	1
IA38	2.75	0.8	—	53	30	3	25
IA52	2.75	5.9	3.56	33	30	6	87
IA48	2.82	6.6	3.44	25	38	4	86
IA31	2.84	0.0	3.55	34	11	6	1
IA42	2.85	0.0	3.42	29	27	9	0
IA59	2.85	4.6	—	62	120	56	4
IA11	2.87	2.9	3.66	54	30	54	53
IA33	2.87	3.8	3.62	42	72	136	81
IA37	2.90	0.0	—	38	20	8	0
IA23	3.09	5.6	3.50	39	13	14	10
IA 4	3.13	1.0	3.87	38	—	—	—
IA55	3.26	6.0	3.58	37	38	4	92
IA60	3.28	10.5	3.46	38	50	19	100
IA27	3.42	10.9	3.43	49	32	8	100
IA40	3.59	6.0	3.69	78	71	55	89
Average	2.36	3.1	—	34	35	30	47
Maximum	3.59	10.9	4.73	78	120	755	100
Minimum	1.15	0.0	3.42	17	10	2	0

Effect of Commercial Starters

A lot of fresh cream of the same history of that described in Table 3 was divided into several 2 liter portions. Each portion (except controls) was pasteurized at 68°C. for 25 minutes. Controls were churned and analyzed at once and again after standing one day at room temperature. All other portions were inoculated with commercial starters, six different brands being used. After the acidity of the cream had been determined it was churned into butter, and determinations were made as shown in the table on page 498.

It is evident that cream inoculated with commercial starters, even when allowed to react for an extra long time, does not show significant changes in indole, acidity of fat, or mold.

II.—BUTTER EXPERIMENTS

Composition of Commercial Butter

The butter (Table 7) was collected in creameries that were operating under good sanitary conditions. Every can of cream was examined and no organoleptic decomposition was noted. It is not impossible that some of the cans contained some decomposed cream whose odor was masked

SAMPLE NUMBER	STARTER	STORAGE AT 22–27° C.	CREAM	BUTTER		
			ACIDITY (LACTIC)	ACIDITY FAT	INDOLE (EXTRACTION METHOD)	MOLD
		days	*per cent*	*cc. 0.1 N*	*gamma per 50 cc. fat*	*per cent*
L1a(a)	none	0	0.18	12	2	0
L1b	none	0	0.18	12	2	0
L1c(a)	none	1	0.22	12	2	0
L1d	none	1	0.21	12	2	0
L1e	E	1	0.97	14	2	0
L1f	F	1	0.94	15	3	0
L1g	G	1	0.97	14	2	0
L1h	H	1	0.93	14	2	0
L1i	I	1	0.90	14	3	0
L1j	J	1	0.96	14	3	0

(a) Not pasteurized.

when it was mixed with fit cream. Some of the cream was factory separated but most was high-grade cream, separated on the farm.

Table 8 contains samples of butter collected at random in the open market. Table 9 shows a few samples collected in a creamery found to be using a high percentage of decomposed cream.

Effect of Storage of Butter

Tables 10, 11, and 12 cover freshly manufactured commercial butter stored at various temperatures. The series of samples in each sequence was from the same churning. Conditions of storage were designed to duplicate commercial practices and, in the case of Table 12, to exceed them in severity. Soon after manufacture commercial butter is stored or shipped at temperatures around freezing. If it is to be stored for any length of time, it is placed in a freezer at approximately −26°C. The experiments indicate no effect on indole or mold content when butter is stored up to about 30 days at room temperature. There is a slow increase in acidity of fat on storage at room temperature. With storage at either refrigerator temperatures or in a sharp freezer there is no significant change in indole, acidity of fat, or mold.

Subsequent to oral presentation of this paper, the use of the indole method by the dairy industry led to the development of information indicating the desirability of studying the relationship between "weedy" flavor in cream and the apparent indole content of butter made therefrom.* Sufficient work has been done to indicate that butter made from "weedy" cream may yield high results. The significance of this phenomenon is being further investigated.

GENERAL CONCLUSIONS

1. Lactic acid fermentation of cream with pure cultures occasions no increase in indole and only insignificant increases in acidity of fat in the

* Private communication from R. V. Hussong.

TABLE 7.—*86 Samples of commercial butter of good quality*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)		
			EXTRACTION METHOD	DISTILLATION METHOD	ALCOHOLIC DILUTION METHOD
	per cent	*cc. 0.1 N*			
KA1	0	8	3		2
KA2	0	8	3		2
KA3	1	8	4		3
KA4	0	8	3		2
KA5	0	8	3		3
KA6	0	8	4		3
KA7	15	9	3		2
KA8	0	6	5		4
KA9	0	7	3		2
KA10	0	5	3		2
KA11	1	6	3		2
KA12	0	8	3		2
KA13	0	—	4		3
KA14	2	—	3		2
KA15	0	—	4		4
KA16	0	7	4		3
KA17	3	10	3		2
KA18	0	10	(a)	3	
KA19	9	11	7		5
KA20	0	7	3		3
KA21	0	6	3	2	
KA22	0	7	2		2
KA23	0	6	3		3
KA24	18	25	4		2
KA25	0	12	5		4
KA26	6	11	5		3
KA27	0	12	6		4
KA28	3	13	6		5
KA29	1	7	(a)	2	
KA30	0	6	4		5
KA31	0	6	5		3
KA32	0	9	5		3
KA33	0	6	3		2
KA34	0	4	4		2
KA35	0	4	3		2
KA36	0	6	3		2
KA37	2	5	(a)	3	
KA38	0	6	4	—	
KA39	0	6	5	—	
KA40	13	7	5	—	
KA41	1	8	3	2	
KA42	0	5	5	4	
KA43	0	6	5	3	
KA44	1	7	4	3	
KA45	53	13	4	2	

(a) Butter contained yellow AB or yellow OB.

TABLE 7.—*Continued*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)		
			EXTRACTION METHOD	DISTILLATION METHOD	ALCOHOLIC DILUTION METHOD
	per cent	*cc. 0.1 N*			
KA46	0	5	5	3	
KA47	0	6	(a)	3	
KA48	0	9	4	3	
KA49	2	6	4	2	
KA50	2	6	5	3	
KA60	4	9	4	4	
KA61	2	9	(a)	6	
KA62	0	12	(a)	7	
KA63	0	5	6	5	
KA64	3	3	8	6	
KA65	0	4	7	5	
KA66	0	4	7	6	
KA67	0	5	7	5	
KA68	0	5	10	7	
KA69	0	6	(a)	6	
KA70	32	9	9	6	
KA71	0	3	6	4	
KA72	0	7	7	4	
KA73	0	9	6	4	
KA74	0	5	7	5	
KA75	0	5	5	4	
KA76	0	3	6	3	
KA77	2	4	(a)	6	
KA78	12	8	5	4	
KA79	14	7	7	4	
KA80	0	5	6	4	
KA81	0	5	6	3	
KA82	0	5	5	3	
KA83	0	5	6	3	
KA84	0	6	8	4	
KA85	0	6	6	4	
KA86	0	6	7	4	
KA87	0	4	9	6	
KA88	0	8	6	3	
KA201	4	14	7	6	
KA202	56	12	9	6	
KA203	7	7	9	7	
KA204	26	6	6	4	
KA205	4	9	6	4	
KA206	4	9	8	4	
KA207	2	7	8	4	
Average	4	7	5	4	3
Maximum	56	25	10	7	5
Minimum	0	3	2	2	2

TABLE 8.—*119 random market samples of commercial butter*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)	
			EXTRACTION METHOD	DISTILLATION METHOD
	per cent	*cc. 0.1 N*		
KA500	0	4	4	2
KA501	0	4	6	4
KA502	0	5	3	2
KA503	0	4	3	2
KA504	0	8	4	2
KA506	0	8	4	2
KA509	0	14	2	1
KA510	0	10	5	2
KA511	0	7	4	2
KA513	0	9	3	2
KA514	0	8	5	3
KA515	0	7	5	2
KA516	0	7	4	1
KA517	2	8	5	3
KA518	2	8	5	3
KA519	0	8	4	3
KA520	41	11	4	4
KA521	49	11	4	5
KA522	45	21	6	5
KA523	0	5	6	3
KA524	0	7	6	5
KA525	11	7	9	6
KA526	0	38	(a)	10
KA527	0	6	10	8
KA528	2	6	10	7
KA529	11	20	7	5
KA530	34	29	(a)	5
KA531	10	3	5	3
KA532	5	7	22	20
KA533	58	17	7	4
KA534	9	29	6	4
KA535	37	11	5	3
KA536	35	16	4	2
KA537	13	11	5	3
KA538	26	13	5	3
KA539	30	11	5	3
KA540	77	15	6	3
KA541	78	23	5	3
KA542	78	20	7	5
KA543	66	30	9	7
KA544	22	22	11	9
KA545	31	60	(a)	5
KA546	26	15	(a)	3
KA547	0	4	4	2
KA548	0	18	3	2
KA549	0	5	6	4

(a) Butter contained yellow AB or yellow OB.

TABLE 8.—*Continued*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)	
			EXTRACTION METHOD	DISTILLATION METHOD
	per cent	*cc. 0.1 N*		
B2B1	0	7	10	6
B2C1	0	11	5	3
B2D1	67	18	(a)	4
KA550	9	23	5	3
KA551	21	27	10	10
KA552	0	6	5	4
KA553	42	16	5	3
KA554	13	10	6	4
KA555	28	15	8	6
KA556	12	31	6	4
KA557	2	7	(a)	4
KA558	46	13	5	3
KA559	14	7	6	3
KA560	28	31	10	10
KA561	0	6	7	6
KA562	48	16	8	7
KA563	15	14	7	5
KA564	14	13	6	3
KA565	6	10	(a)	2
KA566	23	14	4	3
KA567	92	19	(a)	5
KA568	31	5	21	18
KA569	72	14	6	3
KA570	97	19	(a)	4
KA571	61	13	6	4
KA572	94	21	(a)	6
KA573	46	13	7	3
KA574	41	13	(a)	4
KA575	18	10	(a)	4
KA576	65	29	10	7
KA577	11	19	5	4
KA578	16	31	(a)	3
KA579	1	5	5	2
KA580	9	17	5	3
KA581	10	18	(a)	6
KA582	69	18	9	7
KA583	16	19	6	3
KA584	37	16	(a)	3
KA585	30	12	5	3
KA586	39	35	6	4
KA587	39	35	5	3
KA588	1	14	(a)	2
KA589	53	36	8	6
KA590	24	23	(a)	9
KA591	8	35	(a)	5
KA592	53	20	5	3
KA593	30	35	6	3
KA594	49	19	6	4
KA595	34	4	6	5

TABLE 8.—*Continued*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)	
			EXTRACTION METHOD	DISTILLATION METHOD
	per cent	*cc. 0.1 N*		
KA596	31	15	(a)	3
KA597	30	68	(a)	4
KA598	1	26	(a)	4
KA599	19	18	(a)	3
KA600	26	9	(a)	2
KA601	6	11	6	4
KA602	0	65	4	3
KA603	4	16	(a)	5
KA604	12	15	5	3
KA605	5	8	(a)	3
KA606	2	10	6	3
KA607	0	12	6	4
KA608	1	18	5	3
KA609	0	12	5	4
KA610	0	11	6	4
KA611	0	15	6	4
KA612	0	15	6	5
KA613	0	10	6	5
KA614	0	9	5	3
KA615	0	14	6	4
KA616	1	14	6	4
KA617	1	9	8	6
KA618	0	9	8	6
KA619	3	6	(a)	6
Average	21	16	6	4
Maximum	97	68	22	20
Minimum	0	3	2	1

TABLE 9.—*8 samples of commercial butter from creamery using considerable decomposed cream*

SAMPLE NUMBER	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)	
			EXTRACTION METHOD	DISTILLATION METHOD
	per cent	*cc. 0.1 N*		
KA400	4	37	8	5
KA401	25	43	8	6
KA402	0	29	6	5
KA403	0	14	24	19
KA404	2	26	9	6
KA405	2	22	9	7
KA406	14	7	16	14
KA407	0	13	(a)	8
Average	6	24	11	9
Maximum	25	43	24	19
Minimum	0	7	6	5

(a) Butter contained yellow AB or yellow OB.

TABLE 10.—*Effect on commercial butter of storage at 4°C.*

SAMPLE NUMBER	STORAGE PERIOD	MOLD	ACIDITY FAT	INDOLE (GAMMA PER 50 CC. FAT)	
				EXTRACTION METHOD	DISTILLATION METHOD
	days	*per cent*	*cc. 0.1 N*		
Series B2B					
B2B1	0	0	7	10	6
B2B2	2	0	6	10	6
B2B3	4	0	6	9	6
B2B4	9	0	6	9	6
B2B5	14	0	6	9	7
B2B6	21	0	6	9	7
B2B7	35	0	6	10	7
Series B2C					
B2C1	0	0	11	5	3
B2C2	2	6	11	5	3
B2C3	4	3	11	5	3
B2C4	9	2	11	5	3
B2C5	14	5	11	5	3
B2C6	21	2	11	5	3
B2C7	35	3	11	6	3
Series B2D					
B2D1	0	67	18	(a)	4
B2D2	2	65	18	(a)	4
B2D3	4	66	18	(a)	4
B2D4	9	67	18	(a)	4
B2D5	14	71	18	(a)	4
B2D6	21	60	18	(a)	4

(a) Butter contained yellow AB or OB.

TABLE 11.—*Effect on commercial butter of storage in sharp freezer (−26°C.)*

SAMPLE NUMBER	STORAGE PERIOD	MOLD	ACIDITY FAT	INDOLE (EXTRACTION METHOD)
	days	*per cent*	*cc. 0.1 N*	*gamma per 50 cc. fat*
B1X1	0	0	6	—
B1X2	30	0	5	—
B1X3	60	0	4	2
B1X4	90	0	6	2
B1X5	120	0	6	2
B1X6	150	0	6	3
B1X7	180	0	7	2
B1X8	210	0	6	3
B1X9	240	0	5	2
B1X10	270	0	5	2
B1X11	300	0	6	2

TABLE 12.—*Effect on commercial butter of storage at room temperature (20–30°C.)*

SAMPLE NUMBER	STORAGE PERIOD	MOLD	ACIDITY FAT	INDOLE (EXTRACTION METHOD)
	days	*per cent*	*cc. 0.1 N*	*gamma per 50 cc. fat*
B2A1	0	4	16	3
B2A2	1	4	17	2
B2A3	2	6	18	2
B2A4	4	8	20	2
B2A5	8	2	29	2
B2A6	14	—	33	2
B2A7	30	2	40	2

butter made therefrom. Obviously cream subjected to pure lactic acid fermentation could show no mold content.

2. Decomposition induced by inoculation of sterile cream with decomposed cream effects increases in acidity of fat and mold, and may cause an increase in indole.

3. All indices, for both cream and for the butter made therefrom, increase as commercial cream is exposed for increasing periods to an environment favorable to decomposition. The increase is more rapid the higher the temperature of storage. After extreme decomposition begins these indices may show a slight decrease.

4. In many cases butter made from decomposed cream will show high values for indole, acidity of fat, or mold.

5. No significant change in indole, acidity of fat, or mold was noted in butter under conditions duplicating commercial shipping or storage.

A NEW PROCEDURE FOR DETERMINATION OF FLUORINE BY THE PEROXIDIZED TITANIUM METHOD

By DAN DAHLE (Food Division,* U. S. Food and Drug Administration, Washington, D. C.)

Observations on the determination of fluorine by the peroxidized titanium method indicate certain possible sources of error. Filtration through old papers may cause even distilled water to take on a color easily visible in Nessler tubes. The addition of acid or alkali, particularly alkali, seems to increase the amount of color extracted from the paper. It has also been observed that occasionally samples with a low fluorine content, when distilled by the usual Willard and Winter procedure,[1] give distillates which, after the addition of peroxidized titanium, appear even darker than standards containing no fluorine at all. Thus, distil-

* W. B. White, Chief.
[1] *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).

lates cannot always be depended upon to behave like water solutions. Finally, an error may easily be introduced in the process of the *p*H adjustment. Since increased acidity means a deeper color of the peroxidized titanium, any *p*H difference between sample and standard solutions is a source of error.

It therefore appeared desirable to find means of correcting for, or avoiding, these errors. The remarkable affinity of fluorine for aluminum seemed to offer a solution.

The retarding effect of aluminum salts in the separation of fluorine by distillation with $HClO_4$ was mentioned by Willard and Winter.[1] A quantitative study of this effect was later made by Dahle and Wichmann.[2] These authors[3] also studied the interfering effect of aluminum salts on the determination of fluorine by the peroxidized titanium method. Their results in the latter investigation indicate that the addition of aluminum salts alone to a peroxidized titanium solution has no effect on the color. However, the bleaching exerted by fluorine on this color could be largely counteracted by the addition of aluminum ions. Quantitatively it was noted that 2 mg. of Al at *p*H 1.5 prevented all the bleaching ordinarily caused by 0.04 mg. of fluorine.

It is obvious that if the same amount of peroxidized titanium solution were added to two equal aliquot portions of a sample distillate, the *p*H and the resultant color of the two would be the same and the final color would, of course, be the sum of the color of the peroxidized titanium itself and any inherent color in the distillate, less whatever color was bleached out by the fluorine present. If, now, the bleaching action of fluorine were counteracted in one of the aliquot parts by the addition of aluminum salt, the color difference between the two aliquots should be a measure of the fluorine content irrespective of any inherent color in the original solution, provided it can be shown that the addition of aluminum salt causes no change in *p*H.

EXPERIMENTAL

Since Nessler tubes obviously are not suitable for quantitative determinations by this procedure, preliminary readings of the peroxidized titanium color were made in the neutral wedge photometer designed by Clifford.[4] A Corning No. 53 violet filter was used. (Later a Corning No. 511 violet filter with a Noviol shade No. 038 was found more suitable.) It was also found that the color previously recommended for Nessler tube comparisons (0.35 mg. of TiO_2 per 50 cc.) was too weak. Experiments with varying titanium concentrations showed that 0.70 mg. of TiO_2 per 50 cc. was most satisfactory, and this concentration was used in the subsequent experimental work. Above this optimum concentra-

[1] *Loc. cit.*
[2] *This Journal*, 19, 320 (1936).
[3] *Ibid.*, 16, 612 (1933).
[4] *Ibid.*, 19, 130 (1936).

tion, certain advantages gained by the use of larger amounts of titanium were outweighed by the difficulty in reading dark colors and the attendant eye fatigue.

First the question of *p*H was studied. The earlier studies by Wichmann and Dahle[1] had indicated that for a given amount of fluorine a maximum bleach occurred at *p*H 1.5, or slightly below. A re-determination of the optimum *p*H under the changed conditions was made at *p*H values varying from 0.5 to 1.7, the bleach caused by 0.07 mg. of fluorine being determined. The results appear in Table 1.

TABLE 1.—*Effect of pH on color*

F-CONCENTRATION	*p*H	PHOTOMETER READING (AV.)	DIFFERENCE (BLEACH)
mg./50 cc.		*mm.*	*mm.*
0	1.7	40.52	
0.07	1.7	36.50	4.02
0	1.6	46.07	
0.07	1.6	39.72	6.35
0	1.5	49.62	
0.07	1.5	42.35	7.27
0	1.45	50.55	
0.07	1.45	43.19	7.36
0	1.4	51.35	
0.07	1.4	44.00	7.35
0	1.25	52.90	
0.07	1.25	45.66	7.24
0	1.1	53.80	
0.07	1.1	46.50	7.30
0	1.0	54.92	
0.07	1.0	48.15	6.77
0	0.8	55.19	
0.07	0.8	49.68	5.51
0	0.5	55.93	
0.07	0.5	51.06	4.87

As may be seen (Table 1), a lowering of the *p*H causes a considerable change in color. To illustrate, take the following example based on these results: The photometer reading of a solution containing 0.07 mg. of fluorine, having a *p*H of 1.1, would be recorded as 46.50. At *p*H 1.5 a

[1] *Loc. cit.*

solution of the same fluorine content would be read as 42.35 and a fluorine-free solution as 49.62.

A sample solution containing 0.07 mg. of fluorine and having a *p*H of 1.1 would, therefore, if compared to standards with *p*H 1.5, appear darker than the standard containing 0.07 mg. of fluorine. Its bleach would be recorded as 49.62−46.50 or 3.12 mm. as compared to 49.62−42.35 or 7.27 mm., the standard bleach for 0.07 mg. of fluorine. Its fluorine content, then, would be judged as $3.12/7.27 \times 0.07$ or 0.03 mg. instead of 0.07 mg., *i.e.*, an error of 0.04 mg. or 57 per cent would be caused by a 0.4 unit difference in *p*H between sample and reference standards. With present *p*H equipment differences of 0.02 unit represent the practical limit for accuracy of measurements. This would correspond to a 0.3 per cent error.

While the color of peroxidized titanium solutions varies with *p*H, the bleach caused by 0.07 mg. of fluorine is *the same* for any *p*H within the range 1.1 to 1.5 (cf. Table 1). At lower or higher acidities, however, the bleaching effects show definite decreases. Hence any *p*H between 1.1 and 1.5 may be chosen for color comparisons, provided the sample is compared with an unbleached solution *at the same pH*.

The effectiveness of aluminum nitrate in counteracting the bleaching of fluorine was studied next. Varying quantities of a solution containing 140 grams of $(Al(NO_3)_3 \cdot 9\,H_2O$ per liter were added to solutions containing the same quantities of peroxidized titanium and of fluorine, the latter amount being 0.12 mg. All solutions were made to the same volume and *p*H, and read in the photometer. Results appear in Table 2.

TABLE 2.—*Effect of aluminum nitrate on bleach*

F PRESENT	Al-SOLUTION ADDED	PHOTOMETRIC READING (AV.)
mg.	*cc.*	*mm.*
none	none	36.30
0.12	none	30.22
0.12	0.1	34.16
0.12	0.2	35.46
0.12	0.5	35.98
0.12	1.0	36.32
0.12	1.5	36.25
0.12	2.0	36.24
0.12	5.0	36.34

Apparently 1 cc. of aluminum solution (100 mg. of Al) will satisfactorily inhibit the bleaching effect of 0.12 mg. of fluorine at the *p*H (1.3) used in these experiments.

The effect of the addition of aluminum nitrate on the *p*H of the solution is shown in Table 3. The *p*H determinations were made by means of a Beckman *p*H meter with a glass electrode.

TABLE 3.—*Effect of aluminum nitrate on pH*

SAMPLE NO.	pH WITHOUT Al-NITRATE	pH WITH 1 CC. OF Al-NITRATE	DIFFERENCE
1	1.28	1.29	+0.01
2	1.24	1.25	+0.01
3	1.25	1.23	−0.02
4	1.24	1.23	−0.01
5	1.25	1.25	0
6	1.03	1.01	−0.02
7	1.03	1.03	0
8	1.14	1.12	−0.02
9	1.13	1.13	0
10	1.16	1.16	0

The last two experiments (Tables 2 and 3) show that the addition of 100 mg. of Al as aluminum nitrate has no measurable effect on the *p*H, but that it will inhibit the bleaching ordinarily caused by 0.12 mg. of fluorine. Hence, if there are available two equal aliquot parts of a sample solution, each containing less than 0.1 mg. of fluorine, and to one of them is added 100 mg. of Al (1 cc. of nitrate solution), the two aliquot parts will automatically have the same *p*H and after addition of peroxidized titanium they will show a color difference proportional to their fluorine content.

If it is assumed that these sample aliquots contain 0.07 mg. of fluorine and have a *p*H of 1.1, the photometer readings, according to Table 1, would be—

	mm.
For the solution with the bleach inhibited	53.80
For the bleached solution	46.50
Difference due to the fluorine	7.30

If the reference standards had a *p*H of 1.5 rather than 1.1, the standard bleach for 0.07 mg. of fluorine would be recorded as 49.62—42.35 or 7.27 mm. (*cf.* Table 1). The fluorine content of the sample would be judged as $7.30/7.27 \times 0.07$, or 0.0703 mg. The error due to the *p*H difference of 0.4 unit between sample and standards is, in this case, only 0.4 per cent, which is negligible.

The "aluminum system," therefore, permits a comparison of sample solutions of one *p*H with reference standards of a different *p*H if all the various *p*H values fall within the limits $1.1-1.5$. It also provides an automatic adjustment of the *p*H in the two solutions in which the bleach is determined. Instead of a *p*H adjustment within the narrow limits of 0.02 unit as before, this new system requires only an adjustment to any point within the rather wide range of 1.1 to 1.5.

The effect of temperature on the color of the peroxidized titanium solution was also studied. Table 4 gives the results.

TABLE 4.—*Effect of temperature on color*

TEMPERATURE	PHOTOMETER READINGS		DIFFERENCE
	No F	0.055 mg. F	
°C.	mm.	mm.	mm.
25	38.50	32.85	5.65
12	38.07	32.25	5.82
25	39.70	34.05	5.65
12	39.23	33.44	5.79

Apparently the color of peroxidized titanium solutions, both with and without fluorine, decreases with lowered temperature. This agrees with the observations of Merwin.[1] This effect need only be considered, however, when the changes in temperature are great. In ordinary determinations of fluorine the temperature should not vary more than 1–2° during the time the readings are made. The "temperature error" then would be negligible.

Far more important is the possibility of error from the effect of lowered temperature on the reaction between fluorine and aluminum. At pH 1.1–1.5 the formation of the aluminum-fluorine complex (which may be AlF_3

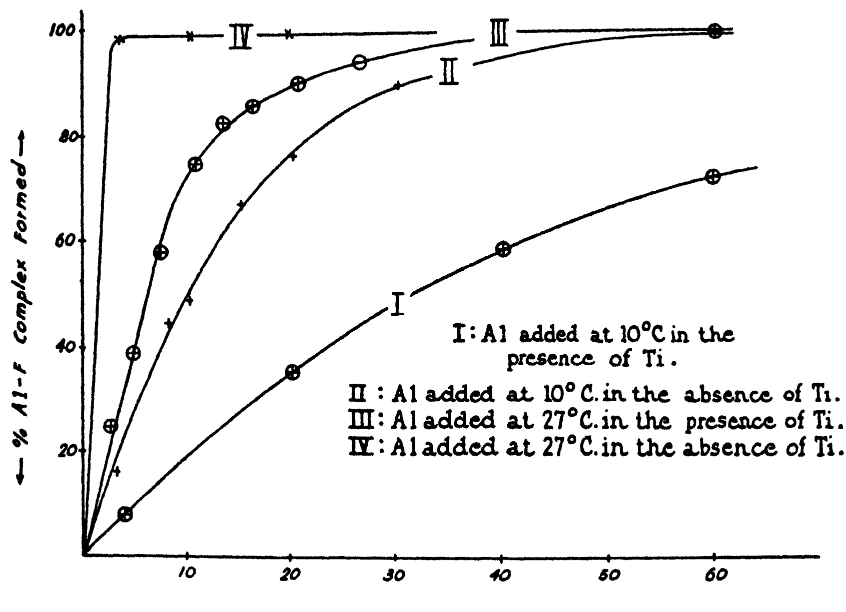

CHART I
FORMATION OF Al-F COMPLEX

[1] *Am. J. Sci.*, 28, 119 (1909).

or AlF_6''') is affected both by temperature and by the presence of titanium ions in the solution. Chart 1 presents the correlation between the time needed for the formation of the aluminum-fluorine complex and such factors as temperature and titanium concentration. It shows that at 27°C. the reaction is practically instantaneous in the absence of titanium and that the lowering of the temperature and/or the introduction of titanium *before* the aluminum is added causes the equilibrium to be reached only gradually. The reaction, incidentally, appears to be of the pseudo-unimolecular type.

Tables 5 and 6 summarize the experimental data used in Chart 1. "Time" means the number of minutes elapsed between the addition of the aluminum solution and the reading in the photometer.

TABLE 5.—*Formation of the Al-F complex in the presence of Ti*

TEMPERATURE	F	Al-SOLUTION	Ti-SOLUTION	TIME	PHOTOMETER READING	Al-F COMPLEX FORMED
°C.	mg.	cc.	cc.	minutes	mm.	per cent
10	0	0	2.0	4	38.38	none
10	0.05	0	2.0	4	32.85	none
10	0.05	0.5	2.0	4	32.94	1.6
10	0.05	0.5	2.0	60	35.83	53.8
10	0.05	1.0	2.0	4	33.04	3.4
10	0.05	1.0	2.0	20	34.68	33.1
10	0.05	1.0	2.0	40	36.00	56.9
10	0.05	1.0	2.0	60	36.61	68.0
10	0.05	2.0	2.0	4	33.55	12.6
10	0.05	2.0	2.0	60	37.75	88.6
10	0.05	5.0	2.0	4	34.31	26.4
10	0.05	5.0	2.0	60	38.40	100.2
27	0	0	2.0	2.5	38.83	none
27	0.05	0	2.0	2.5	33.82	none
27	0.05	1.0	2.0	2.5	35.08	25.2
27	0.05	1.0	2.0	4.5	35.78	39.0
27	0.05	1.0	2.0	7.5	36.74	58.2
27	0.05	1.0	2.0	10.5	37.62	75.8
27	0.05	1.0	2.0	13.5	37.95	82.4
27	0.05	1.0	2.0	16.5	38.13	86.0
27	0.05	1.0	2.0	20.5	38.36	90.6
27	0.05	1.0	2.0	26.5	38.60	95.4
27	0.05	1.0	2.0	60.0	38.83	100.0

The percentage of the aluminum-fluorine complex formed is calculated from the readings showing the amount of bleach inhibited. For example, using the first three lines in Table 5: $38.38-32.85=5.53$ mm.; $32.94-32.85=0.09$ mm.

A neutralization of the bleach corresponding to 5.53 mm. would mean a 100 per cent formation of the aluminum-fluorine complex. Therefore,

0.09 mm. corresponds to 0.09/5.53×100, or 1.6 per cent of the aluminum-fluorine complex formed.

For the experiments listed on Table 6 the aluminum nitrate solution was first mixed with the fluoride, and the titanium solution was added immediately before the reading was taken.

TABLE 6.—*Formation of the Al-F complex in the absence of Ti*

TEMPERATURE	F	Al-SOLUTION	TIME	PHOTOMETER READING	Al-F COMPLEX FORMED
°C.	mg.	cc.	minutes	mm.	per cent
10	0	0	3	37.94	none
10	0.05	0	3	32.94	none
10	0.05	1.0	3	33.36	16.7
10	0.05	1.0	8	34.92	45.2
10	0.05	1.0	10	35.14	49.1
10	0.05	1.0	15	36.16	67.8
10	0.05	1.0	20	36.68	77.2
10	0.05	1.0	30	37.40	90.2
10	0.05	1.0	60	37.94	100.0
27	0	0	3.5	38.83	none
27	0.05	0	3.5	33.82	none
27	0.05	1.0	3.5	38.80	99.4
27	0.05	1.0	10	38.79	99.3
27	0.05	1.0	20	38.81	99.5

To test the practical constancy of the bleaching effect of fluorine on the color of a peroxidized titanium solution regardless of extraneous colors, slightly colored distillates were used. Aliquot portions, to which known quantities of fluorine were added, were treated with titanium and peroxide in the usual manner and compared in the photometer with similar solutions, where distilled water was substituted for the colored distillate. The results are shown in Table 7.

TABLE 7.—*Colored vs. colorless solutions*

SOLUTION	F ADDED	PHOTOMETER READING	DIFFERENCE CORRESPONDING TO 0.01 mg. OF F
	mg.	mm.	mm.
Colorless	none	60.34	
Ditto+Al	none	60.34	
Colorless	0.04	55.40	1.23
Colorless	0.06	52.96	1.23
Colorless	0.08	50.94	1.18
Colored	none	61.43	
Ditto+Al	none	61.41	
Colored	0.04	56.52	1.23
Colored	0.06	53.97	1.24
Colored	0.08	52.12	1.16

From these results (Table 7) it appears that the change in color for a given amount of fluorine is the same in both solutions. It is also verified that the addition of aluminum nitrate causes no color change in the absence of fluorine.* Finally, within the limits 0–0.06 mg. of fluorine, the variation in color appears to be directly proportional to the amount of fluorine present.

The work described was performed with a photometer equipped with a neutral wedge with a density gradient of 0–2. Since a 0–1 density wedge would give greater differences in the scale readings for a given amount of fluorine, its use would be advantageous unless the variations among the individual readings were to show a corresponding increase. Twenty readings were taken with each wedge on each of two peroxidized titanium solutions, one of which contained no fluorine and the other 0.06 mg. Table 8 gives the results, which indicate a superiority of the 0–1 density wedge. However, a statistical treatment of the various sets of readings (omitted for the sake of brevity) indicates approximately the same probable error with both wedges. The type to use, therefore, would be optional.

TABLE 8.—*Effect of the neutral wedge*

WEDGE DENSITY	F	READINGS (AV.)	MAXIMUM	MINIMUM	MAXIMUM VARIATION
	mg.	*mm.*	*mm.*	*mm.*	*mm.*
0–1	none	114.60	115.6	113.9	1.7
0–1	0.06	100.72	101.4	99.9	1.5
0–2	none	62.67	63.0	62.0	1.0
0–2	0.06	54.60	55.0	53.8	1 2

Finally, the procedure was put to a test on solutions containing added amounts of fluorine, unknown to the analyst. Table 9 shows the results obtained by two analysts, experienced in the use of the photometer and in colorimetric measurements. An accuracy of 0.001 mg. is perhaps greater than could be generally expected from the system.

PROCEDURE

REAGENTS

(a) *Aluminum nitrate solution.*—Dissolve 140 grams of $Al(NO_3)_3 \cdot 9\ H_2O$ in 1 liter of water.

(b) *Standard fluoride solution.*—1 cc. = 0.02 mg. of fluorine. Prepare by diluting a stock solution containing 1 gram of fluorine per liter. (For the stock solution NaF with a purity of 98–99% may be weighed out directly, 2.22 grams to a liter.)

(c) *Titanium solution.*—1 cc. = 0.35 mg. of TiO_2. Titanous chloride solution ($TiCl_3$) may be used. Make a suitable amount to 1 liter with 200 cc. of HNO_3 (1+9) and water.

(d) *Hydrogen peroxide.*—6% solution. Dilute 20 cc. of a 30% H_2O_2 to 100 cc. with water.

* Later experiments with an improved photometer indicate that the addition of 1 cc. of aluminum nitrate solution causes a slight increase in the photometer reading. The plus error thus introduced seems to be 0.001 mg. of fluorine or less. In most cases this is negligible.

TABLE 9.—*Quantities of fluorine recovered*

SAMPLE NO.	ANALYST A				ANALYST B			
	READINGS AV.	F FOUND	F ADDED	ERROR	READINGS AV.	F FOUND	F ADDED	ERROR
		mg.	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*
1	64.48	0.006	0.006	0	65.35	0.006	0.006	0
2	64.55	0.006	0.007	−.001	65.09	0.008	0.007	+.001
3	59.75	0.043	0.047	−.004	60.35	0.046	0.047	−.001
4	60.96	0.033	0.032	+.001	62.27	0.031	0.032	−.001
5	63.05	0.017	0.016	+.001	64.24	0.015	0.016	−.001
6	62.01	0.025	0.024	+.001	63.26	0.023	0.042	−.001
7	59.82	0.041	0.040	+.001	61.27	0.039	0.040	−.001
Sample +Al	65.31				66.14			
Standard No F	63.39				63.71			
Standard 0.05F	56.78				57.43			

(e) *Nitric acid* (*1+9*).—Dilute 50 cc. of concentrated HNO_3 to 500 cc. with water.

APPARATUS

(1) *Neutral wedge photometer.*—Described by Clifford and Wichmann.[1]

(2) *Color filter.*—Corning No. 511 violet with or without Noviol shade No. 038, depending on the type of wedge used.

(3) *Glass cells.*—4-inch cell, holding 15–25 cc.

(4) *Volumetric flasks or Nessler tubes.*—50 cc. volume. Check Nessler tubes for volume and discard tubes showing more than 0.3 cc. variation from 50 cc.

(5) *p*H *comparator.*—Block comparator with meta cresol purple, acid cresol red, or any other suitable indicator covering the range 1.1 to 1.5.

DETERMINATION

(1) *Color solutions*

(a) *Standard zero.*—Make 2.00 cc. of titanium solution, 2 cc. of peroxide, and 2 cc. of HNO_3 (1+9) to *exactly* 50 cc.

(b) *Standard 0.05 F.*—Make like standard zero, but add 2.5 cc. of standard F solution (0.05 mg. of F) before making to volume.

(c) *Sample X.*—Use an aliquot portion of distillate containing 0.05 mg. of F (±0.005 mg.) whenever possible, or smaller quantities when necessary. Add 2.00 cc. of titanium solution, 2 cc. of peroxide, and 2 cc. of HNO_3 (1+9) and make to *exactly* 50 cc.

(d) *Sample zero.*—Use an aliquot portion of distillate, equal to that used in Sample X, add 1 cc. of aluminum nitrate solution, *and mix by inverting* the tube or flask *at least 7 times*. Add 2 cc. of peroxide, 2 cc. of HNO_3 (1+9) and 2.00 cc. of titanium solution. Make to *exactly* 50 cc.

Test the *p*H of one standard and one sample solution to see that they fall within the range 1.1–1.5.

(2) *Color comparison*

Before making comparisons check the following:

Color filter.—Must be securely fastened in holder.

Neutral wedge.—Must be fastened so that it cannot slide.

[1] *Loc. cit.*

Glass cell.—Must be thoroughly washed out, particularly if a solution containing aluminum has been used in it before. In placing the cell in the photometer, *always keep it in the same position* to prevent errors resulting from possible non-parallelism of the end surfaces.

Take 10–15 readings on each solution, noting any possible drop after the first few readings. As the eye sometimes requires a slight time for adjustment to the color, the accuracy of the determination will be increased if readings taken during this adjustment period are discarded. Rest eyes frequently by looking at some yellow or light tan surface.

From the averages of the readings calculate mg. of fluorine (F) per sample solution as follows:

$$\frac{\text{Sample zero} - \text{Sample X}}{\text{Standard zero} - \text{Standard } 0.05 \text{ F}} = \frac{\text{F}}{0.05}.$$

APPLICATIONS

The determination of fluorine in the presence of rather large amounts of chlorides, as for example in sea water, has been a problem to which none of the present methods has been directly applicable. The method of Thompson and Taylor[1] necessitates a preliminary chlorine determination and the use of an artificial salt mixture. The use of silver sulfate for precipitation of chlorides was suggested by Kenworthy.[2] Silver perchlorate has been suggested by Wichmann[2] and Armstrong.[3] The writer has used refluxing in the presence of permanganate and sulfuric acid, which changes the major portion of chloride ion into volatile chlorine gas, which is not held back by the reflux condenser. This method, however, is not safe in the presence of ammonium salts, since the highly explosive nitrogen trichloride (NCl_3) may be formed. The objection to all precipitation methods for chlorine, in connection with fluorine determination, is that only small samples (1–2 grams of NaCl) can be handled.

By the use of the aluminum system described, however, the determination can be made directly. A 4 per cent solution of sodium chloride containing 1 p.p.m. of fluorine gave the following readings on 40 cc. aliquot parts:

Sample X :30.36	Standard 0.05 F:29.00
Sample zero:34.43	Standard zero :33.95

The calculation would give 0.0412 mg. of fluorine per 40 cc. or 1.03 p.p.m. in the salt solution as compared to the 1.00 p.p.m. added.

As a rapid qualitative test for fluorine in water supplies, the system can be applied with Nessler tubes alone. With the photometer, of course, it can be made quantitative.

In a recent publication, Sanchis[4] gave a list of interfering ions and the limits below which his method could be applied without causing appreciable errors. The writer has tried the aluminum system in the presence of 2–10 times these quantities of interferences and found it to give correct results (well within 0.1 p.p.m.). Table 10 gives these results.

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[1] *Ind. Eng. Chem. Anal. Ed.*, **5**, 87 (1933).
[2] Private communications.
[3] *Ind. Eng. Chem. Anal. Ed.*, **8**, 384 (1936).
[4] *J. Am. Water Works Assn.*, **28**, 1456 (1936).

TABLE 10.—*Influence of interfering ions*

INTERFERING ION	QUANTITY PRESENT	FLUORINE ADDED	FLUORINE FOUND	ERROR
	p.p.m.	*p.p.m.*	*p.p.m.*	*per cent*
Cl	24000	1.00	1.03	3
SO_4	1000	1.00	1.02	2
HCO_3	1000	1.00	0.99	1
Na	15500	1.00	1.03	3
Ca	1000	1.25	1.27	2
Mg	1000	1.25	1.26	1
Cu	4250	1.00	1.00	0
SiO_2	500	1.00	0.99	1
H_2S	150	1.00	1.01	1
P_2O_5	50	1.00	0.98	2
B	20	1.00	1.00	0
Fe	12	1.00	0.99	1
Fe	25	1.00	0.85	15
Al	4	1.00	0.22	78
Al	0.75	1.00	0.65	35

In most cases the quantity figures given for interference (Table 10) represent the largest concentration tried rather than an upper limit of the allowable concentration.

With the exception of iron and aluminum, none of the ions that ordinarily interfere in methods for determination of micro-quantities of fluorine will cause errors in the aluminum system procedure unless present in amounts much larger than those tolerated in any other method for this determination. Even the iron interference is less here than in other systems. The greater interference of aluminum is to be expected since the success of the proposed system is built on the completeness of the reaction between fluorine and aluminum.

SUMMARY

A new procedure for making fluorine determinations with the peroxidized titanium method is presented. A neutral wedge photometer is used for the colorimetric comparison and the effectiveness of aluminum salts to inhibit the bleaching ordinarily caused by fluorine in a solution of peroxidized titanium is utilized. Experimental data pertaining to the development of the procedure, as well as typical results and applications, are given.

ACKNOWLEDGMENTS

The author is indebted to H. J. Wichmann and Paul A. Mills of the U. S. Food and Drug Administration; to the former for general suggestions in connection with the work, and to the latter for the *p*H comparisons listed in Table 3.

ASCORBIC ACID CONTENT OF FLORIDA CITRUS FRUITS

By L. M. BEACHAM and V. B. BONNEY (U. S. Food and Drug Administration, Washington, D. C.)

During the winter of 1936–37, the Food and Drug Administration made extensive studies on the maturity of oranges. While early results showed that the ascorbic acid content indicated by the reducing value of juice changes surprisingly little as the orange matures, determinations of that substance were continued throughout because of the general interest in the Vitamin C content of citrus fruits.

The determinations cover a wide range of varieties, root stocks, picking dates, and geographic locations. No attempt was made to draw conclusions as to the effect, if any, of these variables on the ascorbic acid content, but it is hoped that the data will be useful to other workers in this field.

Thirty-eight orange groves, each consisting of a single variety, were selected in different parts of Florida. Arrangements were made with the grower in each case so that two oranges from each of 25 marked trees could be taken at regular intervals up to the time of picking. When the grove was finally picked, the fruit was left on one of the 25 trees that had been previously marked, and samples of 50 oranges each were taken from that tree for some time after the regular picking.

Sampling was usually done at 2-week intervals, and was started while the oranges were very immature. With the exception of the Valencias, which were just reaching maturity when the work was closed, sampling was continued for some time after full maturity. In addition to the fruit from these 38 groves, many samples of citrus fruits of different varieties, which had been collected by the Bureau of Plant Industry in connection with their regular work on citrus fruits, were examined.

The writers wish to express their thanks to the Bureau of Plant Industry for the use of their laboratory space, and to John R. Winston, Senior Horticulturist of the Orlando Station, and Paul L. Harding of that Station, for their cooperation and assistance.

METHODS OF DETERMINATION

The juice was extracted by hand squeezing and strained to remove seeds and coarse pulp. Ascorbic acid was determined on the composite juice of 20–25 oranges by the method described by Bessey and King (1933),[1] the juice being titrated with 2.6 dichlorophenol-indophenol solution that had been standardized against commercial crystalline ascorbic acid. Comparison of this acid with the international standard acid showed it to check very closely. It was found that the ascorbic acid solu-

[1] *J. Biol. Chem.*, 103, 687 (1933).

tion changed so rapidly that it was necessary to make a new solution each time a new dye solution was standardized. The dye solution was much more stable, but since the titrations were made at weekly intervals, a new solution was made for each set of determinations.

In addition to the titration with the dye solution, frequent checks were made by an iodometric titration according to the method described by A. J. Lorenz in an unpublished paper on the Vitamin C content of oranges presented at the American Chemical Society meeting in Pittsburgh in September, 1936. In this method 20 cc. of orange juice, to which 4 cc. of concentrated sulfuric acid has been added, is used. An excess of 0.01 *N* iodine solution is added, starch paste being used as indicator. An excess of 0.01 *N* sodium thiosulfate is then added, and back titrated with the 0.01 *N* iodine to a light blue end point. This method gave good checks with the theoretical on the international ascorbic acid. When direct titration was attempted, 0.01 *N* iodine solution being added until a blue color just appeared, Lorenz's conclusion that the results were always somewhat higher than the theoretical and the end point was obscure was confirmed.

RESULTS

In Table 1 are shown the results obtained on the 38 groves. In addition to the milligrams of ascorbic acid per cc. of juice obtained at each picking, there is included a column giving the approximate amount per orange. The amount of juice obtained by hand squeezing was not recorded, but in addition to the oranges examined for ascorbic acid content, the juice was extracted with an electric reamer from 25 of the 50 oranges composing the sample and the average juice per orange was determined. This average figure was used in calculating the number of milligrams of ascorbic acid present in each orange.

Table 2 gives the milligrams of ascorbic acid per cc. and the total milligrams of ascorbic acid in the juice from each of 25 oranges of the Indian River variety, all picked November 16 from one grove, three months before it was picked commercially. The amount of juice extracted from each orange was recorded, so that the results for milligrams of ascorbic acid per orange are actual and not calculated.

Table 3 gives the results of determination of the ascorbic acid content of a large number of samples of Florida orange juice, covering several varieties and root stocks. These samples were taken in different sections of Florida and at different dates, as noted. In some instances samples were taken at different dates from the same grove; in other groves only one sample was taken. As these oranges were hand squeezed, and no record was made of the total juice content, no approximation of the amount of ascorbic acid per orange can be made.

Table 4 gives the results of determination of ascorbic acid of Florida grapefruit juice, lemon juice, and a number of other miscellaneous citrus fruit juices, obtained by hand squeezing.

TABLE 1.—*Ascorbic acid (vitamin C) content of Florida oranges, 1936–37 (results expressed in milligrams)*

GROVE NUMBER	VARIETY	ROOTSTOCK	LOCATION	PERIOD PICKED: OCT. 4–17 PER CC.	OCT. 4–17 PER ORANGE (APPROX.)	OCT. 18–31 PER CC.	OCT. 18–31 PER ORANGE (APPROX.)	NOV. 1–14 PER CC.	NOV. 1–14 PER ORANGE (APPROX.)	NOV. 15–28 PER CC.	NOV. 15–28 PER ORANGE (APPROX.)
2	Parson Brown	Rough Lemon	Winter Haven	.45	35	.37	32	.40	35	.33	31
4	" "	" "	" "	.51	45	.48	47	.46	52	.48	54
7	" "	Sour Orange	Homeland	.64	53	.61	48	.60	45	.57	43
10	" "	Grapefruit	Wauchula	.51	43	.50	43	.45	44	.48	47
12	" "	Rough Lemon	Waverley	.48	46	.46	41	.46	49	.48	51
13	" "	Sour Orange	Lady Lake	.59	60			.60	55	.59	54
15	" "	" "	Oklawaha	.51	49			.49	50	.50	52
18	" "	" "	Citra	.48	37			.49	44	.46	38
19	" "	" "	Crescent City	.52	46			.49	53	.46	47
22	" "	" "	DeLand	.60	52			.60	52	.55	42
5	Pineapple	Rough Lemon	Winter Haven	.58	47	.53	43	.54	46	.55	45
8	"	Sour Orange	Homeland	.70	57	.66	54	.65	53	.71	60
17	"	" "	Citra	.46	33			.46	39	.43	34
24	"	" "	Mims	.65	64			.62	60	.61	63
25	"	" "	Merritt Island	.70	59			.70	63	.73	71
27	"	" "	Ft. Pierce	.51	45			.50	46	.54	52
23	Indian River	" "	Mims								
26	" "	" "	Merritt Island	.60	51			.57	52	.57	56
28	" "	" "	Ft. Pierce	.46	45			.43	44	.47	47
1	Seedling		Haines City	.59	49	.55	51	.52	54	.55	53
3	"		Winter Haven	.46	40	.40	36	.41	36	.38	32
6	"		Homeland	.51	50	.50	47	.44	45	.47	52
9	"		Wauchula	.49	48	.49	41	.50	53	.47	50
11	"		Waverley	.54	49	.59	54	.49	50	.55	56
14	"		Oklawaha	.50	47			.49	54	.46	49
16	"		Oklawaha	.50	49			.54	57	.51	53
20	"		Crescent City	.47	41			.43	45	.43	43
21	"		DeLand	.51	39			.49	39	.46	35
29	Temple	Sour Orange	Winter Garden							.65	79
30	Valencia	" "	Ft. Pierce								
31	"	" "	Homeland								
32	"	Rough Lemon	Lake Placid								
33	"	" "	Avon Park								
34	"	" "	Winter Haven								
35	"	Sour Orange	Bonaventure								
36	"	" "	Winter Haven								
37	"	Rough Lemon	Winter Haven								
38	"	Sour Orange	Lady Lake								

TABLE 1.—*Continued*

PERIOD PICKED (*Continued*)															
NOV. 29–DEC. 12		DEC. 13–26		JAN. 3–16		JAN. 17–30		JAN. 31–FEB. 13		FEB. 14–27		FEB. 28–MAR. 13		MAR. 14–27	
PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)	PER CC.	PER ORANGE (APPROX.)
.33	35	.33	33												
.44	50	.40	41												
.59	45	.50	39			.59	47			.51	37	.60	46		
.47	47	.51	51												
		.46	55			.50	56								
		.59	67	.50	53	.54	58	.53	54	.56	57	.55	58	.45	46
		.48	60			.38	40								
		.48	51			.42	39								
.51	60	.55	59			.56	57								
		.59	51	.59	53	.60	53	.53	47	.58	49	.62	50	.55	50
.51	45	.57	50	.54	51	.56	50			.55	48				
.71	63	.66	63	.60	64	.56	59	.59	59			.55	52	.59	62
.46	35	.46	41	.42	38	.44	40	.49	44	.50	43	.49	45	.45	44
.59	57	.63	62	.59	64	.68	75	.61	66	.61	69				
.68	66	.70	69	.69	85	.74	82	.65	71	.61	67	.57	59	.65	69
.53	48	.57	56	.54	55	.53	58	.54	55	.55	62	.49	50	.58	66
.51	44	.44	43	.43	43	.48	53	.41	41	.45	45	.44	45	.45	46
.52	53	.57	58	.59	62	.56	66	.55	62	.56	71	.55	62	.63	66
.47	49	.51	56	.47	50	.46	51	.44	49	.49	49	.36	36	.41	39
.61	58	.55	66	.55	71										
.40	36	.43	39												
.49	53	.48	54	.45	52	.46	56	.46	47	.42	43	.45	45	.44	46
.51	53	.48	54	.47	47	.50	50	.49	45	.53	50				
.55	54	.53	57	.56	62	.59	69	.56	72			.60	67		
.41	50	.42	53	.42	52	.41	46	.44	50	.42	46	.42	46	.40	40
.48	58	.51	69			.50	65								
.46	49	.44	46	.42	49	.42	55	.43	52	.42	48	.44	50		
.46	38	.48	42	.42	38	.50	45	.47	38	.53	43	.52	40		
		.56	81					.61	81	.55	89	.60	91	.55	86
.59	63			.51	63			.49	65	.49	72	.45	72	.49	66
.51	48			.53	53			.55	58	.51	56	.57	67	.49	58
.55	59			.51	55			.51	60	.47	54	.47	59	.49	67
.47	50			.48	59			.45	53	.46	56	.47	56	.45	60
.46	44			.46	52			.47	54	.45	50	.47	57	.44	56
.62	73			.56	80			.53	81	.50	76	.52	84	.55	79
.49	49			.46	52			.44	51	.43	49	.45	54	.41	50
.41	46			.39	44			.35	39	.35	42	.37	43	.33	38
				.56	66			.53	68	.56	72	.52	69	.43	57

TABLE 2.—*Variations in ascorbic acid content of individual oranges picked Nov. 16, 1936, from Grove 28*

JUICE	PER CC.	PER ORANGE
cc.	mg.	mg.
101	.42	42
102	.42	43
91	.54	49
90	.49	44
93	.44	41
87	.39	34
90	.41	37
112	.36	40
99	.39	39
91	.41	37
113	.41	46
104	.34	35
108	.36	39
86	.51	44
112	.44	49
91	.44	40
104	.47	49
123	.42	52
96	.42	40
102	.39	40
92	.42	39
99	.42	42
106	.51	54
98	.44	43
105	.32	34
Av. 99.8	.42	42

TABLE 3.—*Ascorbic acid (vitamin C) content of Florida oranges, 1936–37*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
39	Orlando	11– 7–36	Seedling		.51
"	"	11–14–36	"		.53
"	"	11–28–36	"		.53
"	"	1– 9–37	"		.44
"	"	1– 9–37	"		.48
"	"	1– 9–37	"		.56
"	"	1–23–37	"		.36
"	"	1–23–37	"		.40
"	"	1–23–37	"		.40
"	"	1–23–37	"		.40
"	"	1–23–37	"		.43
"	"	1–23–37	"		.43
"	"	1–23–37	"		.46

TABLE 3.—*Continued*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
39	Orlando	1-23-37	Seedling		.50
"	"	1-23-37	"		.50
"	"	1-23-37	"		.50
"	"	1-23-37	"		.50
"	"	1-23-37	"		.66
"	"	2-13-37	"		.51
40	Plymouth	12-22-36	"		.59
41	"	12-22-36	"		.48
42	"	12-22-36	"		.55
43	Florence Villa	11-28-36	"		.53
"	"	2-27-37	"		.37
"	"	2-27-37	"		.40
"	"	2-27-37	"		.40
"	"	2-27-37	"		.40
"	"	2-27-37	"		.40
"	"	2-27-37	"		.40
"	"	2-27-37	"		.40
"	"	2-27-37	"		.42
"	"	2-27-37	"		.42
"	"	2-27-37	"		.45
44	Citra	12-22-36	Pineapple	Sour Orange	.55
"	"	2- 6-37	"	" "	.51
"	"	2-27-37	"	" "	.45
"	"	2-27-37	"	" "	.48
"	"	3-13-37	"	" "	.52
45		12-22-36	"	" "	.70
46		2-27-37	"	" "	.64
47		2-27-37	"	" "	.50
48		2-27-37	"	" "	.53
49	Florence Villa	2-13-37	"	Rough Lemon	.49
50	"	2-13-37	"	" "	.49
51	"	2-13-37	"	" "	.49
52	"	2-13-37	"	" "	.51
53	Orlando	11- 7-36	"	" "	.51
"	"	12-12-36	"	" "	.54
"	"	1-23-37	"	" "	.46
"	"	2-13-37	"	" "	.47
54	Plymouth	12-22-36	"	" "	.64
55	"	12-22-36	"	" "	.68
56	"	12-22-36	"	" "	.53
57	Florence Villa	2-13-37	"	Sweet Orange	.40
58	Citra	12-22-36	Parson Brown	Sour Orange	.55
"	"	2- 6-37	" "	" "	.48
"	"	3-13-37	" "	" "	.52
59	Orlando	1- 9-37	" "	" "	.54
"	"	1-23-37	" "	" "	.53
"	"	2- 6-37	" "	" "	.55

TABLE 3.—*Continued*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
60	Orlando	11- 7-36	Parson Brown	Sour Orange	.57
"	"	11-21-36	" "	" "	.55
"	"	12-12-36	" "	" "	.57
"	"	1-23-37	" "	" "	.53
"	"	2-13-37	" "	" "	.59
61	"	11- 7-36	" "	Rough Lemon	.48
"	"	11-21-36	" "	" "	.50
"	"	12-12-36	" "	" "	.51
"	"	1-23-37	" "	" "	.43
"	"	2-13-37	" "	" "	.40
62	"	12-12-36	Conner's Seedless	Sour Orange	.57
"	"	1-23-37	" "	" "	.53
"	"	2-13-37	" "	" "	.53
"	"	2-27-37	" "	" "	.61
"	"	2-27-37	" "	" "	.50
"	"	2-27-37	" "	" "	.50
"	"	2-27-37	" "	" "	.50
"	"	2-27-37	" "	" "	.56
"	"	2-27-37	" "	" "	.50
"	"	2-27-37	" "	" "	.56
"	"	2-27-37	" "	" "	.50
63	Plymouth	11- 7-36	" "	Rough Lemon	.52
"	"	11-21-36	" "	" "	.50
"	"	12-22-36	" "	" "	.48
"	"	1-23-37	" "	" "	.50
64	"	12-22-36	Homosassa	Sour Orange	.62
65	"	12-22-36	"	Rough Lemon	.44
66	Orlando	12-12-36	"	" "	.51
"	"	1-23-37	"	" "	.43
"	"	2-13-37	"	" "	.40
67	Plymouth	12-22-36	"	Sweet Orange	.42
68	"	12-22-36	"	" "	.59
69	Orlando	12- 4-36	Valencia	Sour Orange	.47
"	"	1-30-37	"	" "	.44
"	"	2-20-37	"	" "	.43
70	"	12- 4-36	"	" "	.63
"	"	1-30-37	"	" "	.54
"	"	2-20-37	"	" "	.53
71	Merritt Island	12- 4-36	"	" "	.53
"	" "	1-30-37	"	" "	.48
"	" "	2-20-37	"	" "	.42
72	Mims	1-30-37	"	" "	.56
"	"	2-20-37	"	" "	.51
73	"	1-30-37	"	" "	.60
"	"	2-27-37	"	" "	.48
"	"	3-27-37	"	" "	.57
74	"	1-30-37	"	" "	.42

TABLE 3.—*Continued*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
74	Mims	2-27-37	Valencia	Sour Orange	.40
"	"	3-27-37	"	" "	.43
75	"	1-30-37	"	" "	.50
"	"	2-27-37	"	" "	.42
"	"	3-27-37	"	" "	.43
76	"	1-30-37	"	" "	.46
"	"	2-27-37	"	" "	.42
"	"	3-27-37	"	" "	.43
77	"	1-30-37	"	" "	.54
"	"	2-27-37	"	" "	.53
"	"	3-27-37	"	" "	.50
78	Wabasso	2- 6-37	"	" "	.60
"	"	3-13-37	"	" "	.55
79	"	2- 6-37	"	" "	.57
"	"	3-13-37	"	" "	.55
80	Wabasso	2- 6-37	"	" "	.40
"	"	3-13-37	"	" "	.36
81	"	2- 6-37	"	" "	.49
"	"	3-13-37	"	" "	.47
82	Orlando	11-14-36	"	Rough Lemon	.54
"	"	12-12-36	"	" "	.49
"	"	1- 9-37	"	" "	.55
"	"	1-23-37	"	" "	.46
"	"	2- 6-37	"	" "	.49
83	"	12- 4-36	"	" "	.45
"	"	1-30-37	"	" "	.40
"	"	2-20-37	"	" "	.42
84	Merritt Island	12- 4-36	"	" "	.47
"	" "	1-30-37	"	" "	.48
"	" "	2-20-37	"	" "	.42
85	Mims	1-30-37	"	" "	.52
"	"	2-20-37	"	" "	.47
86	Homestead	2-13-37	"	" "	.45
87	Redland	2-13-37	"	" "	.47
88	"	2-13-37	"	" "	.47
89	Florence Villa	2-13-37	"	" "	.37
"	" "	3-13-37	"	" "	.42
90	" "	2-13-37	"	" "	.34
"	" "	3-13-37	"	" "	.34
91	Orlando	12- 4-36	"	Grapefruit	.51
"	"	1-30-37	"	"	.44
"	"	2-20-37	"	"	.42
92	Homestead	2-13-37	"	"	.43
93	Redland	2-13-37	"	"	.49
94	"	2-13-37	"	"	.60
95	Orlando	12- 4-36	"	Sweet Orange	.37
"	"	1-30-37	"	" "	.36
"	"	2-20-37	"	" "	.37

TABLE 3.—*Continued*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
96	Orlando	12- 4-36	Valencia	Cleopatra	.55
"	"	1-30-37	"	"	.48
"	"	2-20-37	"	"	.42
97	"	1-30-37	"	Lue Gim Gong	.58
98	Homestead	2-13-37	"	Cleopatra	.47
99	Plymouth	12-22-36	Jaffa	Sweet Orange	.51
100	"	12-22-36	"	" "	.42
101	"	12-22-36	"	" "	.44
102	Winter Garden	11-14-36	"	Rough Lemon	.46
"	" "	11-28-36	"	" "	.45
"	" "	1- 9-37	"	" "	.40
103	Plymouth	12-22-36	Hamlin	" "	.59
104	Orlando	1- 9-37	"	" "	.44
"	"	1-23-37	"	" "	.46
105	"	1-23-37	"	" "	.40
"	"	2-13-37	"	" "	.40
106	"	11-14-36	"16 to 1"	" "	.54
"	"	11-28-36	"	" "	.51
"	"	1- 9-37	"	" "	.46
107	"	11-14-36	Boones Early	" "	.54
"	"	11-28-36	" "	" "	.55
"	"	1- 9-37	" "	" "	.52
108	"	11-14-36	Unknown	" "	.60
"	"	11-28-36	"	" "	.61
"	"	12-12-36	"	" "	.54
"	"	1- 9-37	"	" "	.62
109	"	12- 4-36	Lue Gim Gong	Sour Orange	.61
"	"	12-12-36	" " "	" "	.59
"	"	2-20-37	" " "	" "	.51
110	"	12- 4-36	Enterprise Seedless	Sour Orange	.65
111	Mims	1-30-37	Drake	" "	.50
"	"	2-27-37	"	" "	.61
"	"	3-27-37	"	" "	.53
112	Plymouth	12-22-36	Sweet Seville	Sweet Orange	.46
113	"	12-22-36	Majorca	" "	.46
114	"	12-22-36	Mediterranean Sweet	" "	.55
115	"	12-22-36	" "	Rough Lemon	.62
116	"	12-22-36	Navel	Sweet Orange	.55
117	"	12-22-36	Blood	" "	.48
118	"	12-22-36	"	" "	.51
119	"	12-12-36	Sour Orange Seedling		.30
119	"	1- 9-37	" " "		.31
120	Merritt Island	1- 9-37	" " "		.48
121	" "	1- 9-37	" " "		.36
122	Plymouth	1- 9-37	Bitter Sweet Orange Seedling		.77
123	Merritt Island	1- 9-37	" " "		.64

TABLE 4.—*Ascorbic acid (vitamin C) determinations on miscellaneous Florida citrus fruits, 1936–37*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
124	Wabasso	2– 6–37	Marsh Seedless Grapefruit	Sour Orange	.43
125	"	2– 6–37	" " "	" "	.46
126	"	3–13–37	" " "	" "	.36
127	"	3–13–37	" " "	" "	.42
128	"	2– 6–37	" " "	" "	.36
"	"	3–13–37	" " "	" "	.34
129	Cocoa	12–12–36	" " "	" "	.38
130	Merritt Island	2–20–37	" " "	" "	.39
131	Atwood	3–13–37	" " "	" "	.31
131	Crescent City	12–12–36	" " "	" "	.32
132	Orlando	12–12–36	Common Grapefruit	Rough Lemon	.40
"	"	1– 9–37	" "	" "	.40
133	"	1–23–37	" "	" "	.40
"	"	2– 6–37	" "	" "	.40
"	"	3–20–37	" "	" "	.33
134	"	1–23–37	" "	" "	.40
"	"	2– 6–37	" "	" "	.38
"	"	3–20–37	" "	" "	.33
135	"	1–23–37	" "	" "	.33
"	"	2– 6–37	" "	" "	.33
"	"	3–20–37	" "	" "	.33
136	Plymouth	12–22–36	" "	Unknown	.42
137	Merritt Island	2–20–37	" "	"	.35
138	Atwood	3–13–37	" "	Sour Orange	.39
139	Plymouth	12–22–36	Silver Cluster Grapefruit	Sweet Orange	.50
140	Merritt Island	2–20–37	Pink Foster Grapefruit		.37
141	Ocoee	11–28–36	Temple Orange	Sour Orange	.53
"	"	12–12–36	" "	" "	.54
"	"	1– 9–37	" "	" "	.64
142	Merritt Island	1– 9–37	" "	" "	.72
143	Plymouth	1– 9–37	" "	Rough Lemon	.64
144	"	1– 9–37	" "	" "	.42
145	"	1– 9–37	" "	" "	.51
146	Orlando	11–14–36	Tangerines	" "	.27
"	"	1– 9–37	"	" "	.19
147	"	11–14–36	"	Unknown	.27
"	"	11–28–36	"	"	.30
148	"	11–28–36	Satsuma	Rough Lemon	.28
"	"	1– 9–37	"	" "	.28
"	"	1–23–37	"	" "	.30
"	"	2– 6–37	"	" "	.25
149	Plymouth	1– 9–37	"	" "	.36
150	"	1– 9–37	"	" "	.22
151	"	1– 9–37	King Orange	Sour Orange	.26

TABLE 4.—*Continued*

GROVE NUMBER	LOCATION	DATE PICKED	VARIETY	ROOT STOCK	PER CC.
					mg.
152	Plymouth	1- 9-37	King Orange	Sour Orange	.20
153	Wabasso	2- 6-37	" "	" "	.14
154	Plymouth	1- 9-37	" "	Rough Lemon	.22
155	Florence Villa	2-13-37	" "	" "	.16
156	Plymouth	1- 9-37	Mandarin Orange	Sour Orange	.31
157	"	1- 9-37	" "	" "	.26
158	Orlando	1- 9-37	Sweet Lemon	Rough Lemon	.38
"	"	2-27-37	" "	" "	.45
159	Plymouth	1- 9-37	Everbearing Lemon	" "	.42
160	De Soto City	12-22-36	Perrine Lemon (Green)	" "	.38
"	" " "	12-22-36	Perrine Lemon (Ripe)	" "	.30
"	" " "	1- 9-37	Perrine Lemon (Green)	" "	.35
"	" " "	1- 9-37	Perrine Lemon (Ripe)	" "	.22
161	" " "	12-22-36	Persian Lime	" "	.32
"	" " "	1- 9-37	" " (Green)	" "	.33
"	" " "	1- 9-37	" " (Ripe)	" "	.29
162	Orlando	2-27-37	Rangpur Lime	*	.08
163		12-12-36	Oblong Kumquat	*	.13
164	Orlando	1- 9-37	" "	Rough Lemon	.24
165	"	1- 9-37	Eustis Limequat	Cleopatra	.18
166	Plymouth	1- 9-37	" "	Rough Lemon	.24
167	Orlando	12-12-36	Calamondin	" "	.35
"	"	1- 9-37	"	" "	.34
"	"	1-23-37	"	" "	.33

* Unknown.

DETECTION OF GUMS IN CERTAIN FOOD PRODUCTS

By F. LESLIE HART (U. S. Food and Drug Administration, Los Angeles, Calif.)

The use of edible gums and gum-like substances in prepared foods is increasing. Methods for their detection are of interest to all food chemists, but particularly to the regulatory official whose duty it is to preserve the integrity of food and prevent adulteration. By reason of their ability to increase viscosity or "body" of a food gums may mask the addition of undue amounts of water or other liquid ingredients and thereby serve to conceal inferiority.

One of the most inclusive procedures for detection of gums in foods is that of Cook and Woodman.[1] Proteins are removed with tannic acid and gums are precipitated with acetone and differentiated by the volumes of

[1] *J. Ind. Eng. Chem.*, **10**, 530 (1918).

alcohol necessary to cause precipitation. However, this general procedure is not always applicable to the various food products and gums that may confront the analyst in specific cases. Other precipitants separate protein matter more completely than does tannic acid and do not precipitate locust bean gum, one of the gums now commonly used in food products.

The Association of Official Agricultural Chemists has adopted a method for the detection of gums and dextrin in wines[1] and one for gums in cheese,[2] as reported by Cross.[3] No study has been reported by the Association on the adaptability of either of these methods to foods other than the ones mentioned. Patrick[4] proposed a method for the detection of thickeners in ice cream. This method does not differentiate gums from gelatin or egg protein, and it also fails if the cream has become sour. Wildman[5] developed a microscopic technic for the detection of the insoluble gums karaya and tragacanth in catsup, which may be applicable to other foods. He also applied the iodine color reaction for agar-agar as given in U.S.P. X1 to the detection of this gum in various food products.

The present study was undertaken while the writer was in Food Division,* U. S. Food and Drug Administration, Washington, D. C. The several methods developed are separated under appropriate headings because it was soon discovered that one procedure could not be applied to all types of food products.

DETECTION OF AGAR-AGAR IN MAYONNAISE

Mayonnaise, because of its complex composition and physical character, presents unusual difficulties in the identification of gums by the general procedure of Cook and Woodman. Samples with relatively high egg content usually lead to erroneous conclusions because of incomplete separation of the proteins by the tannic acid precipitant. It was also found that tannic acid, as well as certain other protein precipitants, precipitates agar in whole or in part from its aqueous solutions. Table 1 shows the effect of various precipitants on 15 cc. of a 33 per cent agar solution.

Trichloracetic acid proved to be the most promising precipitant for use in separating the proteins from agar.

Specific identification of the precipitated gum, as distinguished from possible interfering proteins, would assist materially in the interpretation. It is well known that mineral acids readily hydrolyze gums to sugars. According to unpublished work of H. E. Goresline (U. S. Bureau of Chemistry and Soils) agar forms various aldo- and keto-hexoses and

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[1] *Methods of Analysis, A.O.A.C.*, 1935, 169.
[2] *Ibid.*, 295.
[3] *This Journal*, 18, 432 (1935).
[4] U. S. Bur. Chem. Bull. 116, p. 24 (1914).
[5] *This Journal*, 18, 637 (1935).
* Dr. W. B. White in charge.

TABLE 1.—*Effect of various precipitants on 15 cc. of a 33% agar solution*

PRECIPITANT	REACTION
Trichloracetic acid	No precipitate, cold or hot
Potassium ferrocyanide and acetic acid	Precipitate
Dialyzed iron	Precipitate
Picric acid	No precipitate, cold or hot
Phosphotungstic acid	Precipitate cold; dissolved on heating
Tannic acid	Precipitate
Mercuric nitrate	No precipitate cold; precipitate hot

pentoses. After the removal of interfering substances these products of hydrolysis can be identified by Seliwanoff's resorcinol test[1] or Bial's orcinol test[2] for pentoses, and thus the nature of the precipitate separated as gum can be confirmed.

In the development of the technic used there was presented the problem of removing the lower liquid portion containing the gum from a centrifuged mixture in which separation of the material into layers leaves a fatty or emulsified layer at the top. To effect the removal a device* to be inserted in the centrifuge bottle was perfected; it consists of a wide-bore glass tube, flared slightly at the upper end and fitted at the lower end with a ground-glass cap, overall length about one-half inch less than that of the centrifuge bottle. The tube is supported in the neck of the bottle by a slotted rubber stopper. After the material has been centrifuged the pipet is inserted through the tube, the glass cap being pushed off, and the aliquot part desired is removed without disturbing the supernatant layer. The method follows:

SEPARATION AND DETECTION OF GUM

Weigh 50–75 grams of mayonnaise into a 250 cc. beaker, add 40 cc. of hot water, and mix thoroughly. Heat to a temperature of 65–70°C., add 10 cc. of trichloracetic acid solution (50 g./100 cc.), and maintain at a temperature of 65–70°C. for 10 minutes, or until the emulsion shows signs of breaking. Too long a period of heating will hydrolyze any gums present. Transfer the mixture to an 8 ounce nursing bottle, cool, add 30–40 cc. of toluene, mix thoroughly by shaking, insert a Vohale tube in the nursing bottle, and centrifuge for 15–20 minutes at 1,200 r.p.m. By means of a pipet inserted through the Vohale tube, remove as much as possible of the lower aqueous layer and filter through a tight paper into an 8 ounce nursing bottle. Test for complete removal of proteins with 1 cc. of trichloracetic acid solution. If a turbidity ensues, refilter. To the clear filtrate add twice its volume of acetone, mix by shaking, and let stand until the precipitate coagulates somewhat. This precipitate consists of protein material not removed by the trichloracetic acid, gums, dextrin, gelatin, et cetera.

Centrifuge the acetone mixture. Discard the liquid and wash the residue with 70

[1] Browne, Handbook of Sugar Analysis, p. 380.
[2] *Ibid.*, 382.
* To identify this device it has been named the "Vohale Tube," as it was developed as the result of discussion of the problems in Food Division by F. A. Vorhes, H. A. Lepper and the writer.

per cent acetone solution; centrifuge, and again discard the liquid. To the residue add 30–50 cc. of boiling water and 2 or 3 drops of acetic acid and stir vigorously for a few minutes. Filter, if necessary, and transfer the clear solution to a graduated cylinder. Add alcohol, one volume at a time, until a well-defined precipitation occurs. No precipitate after the use of five volumes of alcohol and 2 hours' standing indicates absence of gums and dextrin. (Agar ordinarily requires four volumes or more of alcohol.)

Decant and filter to separate the agar and dissolve it by pouring boiling water through the filter (not more than 30 cc.). Transfer the solution to a 100 cc. beaker, add one-third the volume of concentrated HCl, and heat to incipient boiling to hydrolyze the agar to sugars. Immediately add a few crystals of resorcinol and boil gently for 1 minute. (The immediate development of a deep red color, followed by formation of a red-brown precipitate within 1 minute is confirmatory evidence of the presence of agar.)

NOTES

1. The resorcinol test applied herein is indicative of ketoses. Bial's orcinol test, indicative of pentoses, may also be used, but the precautions given in Browne's text[1] should be observed.

2. Samples with only a small amount of gum may require several hours for separation of the acetone precipitate. The coagulation of both the acetone and the alcohol precipitates may be hastened by the addition of a few crystals of powdered alum.

3. A faint pink or orange color with resorcinol may develop in the absence of gums. An eosin-red color, with subsequent formation of a red-brown precipitate, should develop to confirm the presence of gums. A comparison may be made with the color developed upon heating a solution of 2 mg. of levulose with HCl and resorcinol as outlined.

4. Digestion of the alcohol precipitate with HCl should not be unduly prolonged because of danger of caramelization of the sugars formed by hydrolysis of the gums. With the acid concentration given, heating to incipient boiling will hydrolyze sufficient gum to give a pronounced test.

Negative results were obtained on four samples of gum-free mayonnaise containing 8–12 per cent of egg yolk. An occasional precipitate was obtained with four volumes of alcohol, but no confirmatory color tests were obtained. Occasionally, particularly with high egg content, a fairly heavy acetone precipitate was obtained, which, however, was coagulated by the heating with the dilute acetic acid. The interference of sucrose was tested up to 6 per cent, with negative results.

Positive results (precipitation and color reactions) were obtained on a 50 gram sample of a mayonnaise containing 12 per cent of egg yolk and 0.55 per cent of agar. A mixture of 25 grams of this mayonnaise and 25 grams of agar-free mayonnaise gave well-defined, positive reactions, demonstrating that the method will detect 0.14 gram of agar in 50 grams of mayonnaise. It is believed that amounts as low as 0.1 gram in 50 grams (corresponding to 0.2 per cent) can also be easily detected by the method.

The procedure has been tried only on agar in the absence of other

[1] *Loc. cit.*

gums, dextrins, or pectin. Cook and Woodman give directions for the removal of pectin, and dextrins could, perhaps, be converted into maltose by diastase before the acetone precipitation and thus removed. Further work is necessary to establish the effectiveness of the procedure and the applicability of the color tests to gums such as tragacanth, arabic, karaya, and locust bean.

DETECTION OF ADDED GUMS IN COTTAGE AND CREAM CHEESE

Small quantities of gum, sometimes as low as 0.1–0.05 per cent, are used in the manufacture of soft curd cheeses. Its detection in these products necessitates complete separation of milk proteins, which would otherwise precipitate upon the later addition of alcohol. It was found that the use of trichloracetic acid as a protein reagent resulted in a filtrate that was practically free from precipitable proteins. The method follows:

PREPARATION OF SAMPLE

Various cheeses require different treatment for the separation of the gum solution from the cheese, therefore optional procedures are given.

(1) *Cream cheese or creamed cottage cheese.*—Transfer 100 grams of cheese to a 500 cc. casserole, warm on the steam bath, and macerate with petroleum ether. Decant the ether and repeat the treatment until the ether is colorless. Warm the defatted cheese on the steam bath to remove residual petroleum ether, add 100 cc. of hot water, and bring to a boil, stirring continually. Remove the casserole from the flame, add 4 or 5 drops of ammonia, mix thoroughly, and transfer to a 250 cc. centrifuge bottle or a nursing bottle. Neutralize with concentrated acetic acid, using litmus paper as indicator, and add 4 or 5 drops in excess. Shake thoroughly and centrifuge 10–20 minutes. Separate the liquid either by decantation or by use of the Vohale tube, and proceed as directed under "Separation of Gum"

(2) *Cream cheese that does not separate distinctly into layers upon centrifuging or that separates into a top layer of curd and fat, a middle aqueous layer, and a bottom layer of curd.*—Separate the aqueous gum solution by filtering under vacuum through a coarse filter, using an 11 cm. Büchner funnel. Proceed as directed under "Separation of Gum."

(3) *Cottage cheese (uncreamed).*—Omitting the treatment with petroleum ether, proceed as directed under (1), beginning with " . . . add 100 cc. of hot water . . . ."

SEPARATION OF GUM

Evaporate to about 40 cc. the separated gum solution obtained by centrifuging or filtering (disregard precipitate formed during concentration), add 10 cc. of trichloracetic acid solution (50 g/100 cc.), and hold at 70°C. until the precipitate coagulates (avoid prolonged heating, which will hydrolyze the gum). Cool, and filter clear.

Add 4 volumes of alcohol to the filtrate and allow the mixture to stand 2 or 3 hours, if necessary, to coagulate the separated gum. (A precipitate at this point indicates gums.)

DETECTION OF GUM

Centrifuge the mixture, decant the supernatant liquid, and wash with 70 per cent alcohol. Centrifuge, and again decant or filter. Dissolve the residue in a mini-

mum quantity of hot water, and reprecipitate with 4 volumes of alcohol plus 2 or 3 drops of acetic acid. This reprecipitation removes traces of sugars occluded by the first precipitation.

Allow the precipitate to coagulate, centrifuge, and decant. Dissolve the residue in a minimum quantity of water, transfer to a 50 cc. beaker, and evaporate to 5 cc. Add an equal volume of concentrated HCl, boil 30–60 seconds, and add 2 or 3 crystals of phloroglucinol. A deep amber or cherry red color at this point confirms the presence of gums.

EXPERIMENTAL WORK

The method, as outlined, was tried on mixtures made by adding from 0.05 to 0.10 gram of various gums in aqueous solution to 100 grams of a creamed cottage cheese known to be gum-free. Commercial samples known to contain gum were also examined, and the results are reported in Table 2.

Positive results were obtained from 0.1 per cent mixtures of the following gums when added to gum-free cheese: agar-agar, tragacanth, India gum, karaya, carob bean (locust bean), and gum arabic. Positive tests were also obtained with the following commercial gums: "Neogell," "Hygell," and "Tragon A." To test the delicacy of the separation a mixture was prepared containing 0.05 per cent of carob bean gum, and another containing 0.05 per cent of tragacanth. Positive tests were given in both instances.

Gelatin amounting to 0.2 per cent was added in solution form to a gum-free cheese, and the procedure outlined was followed. A slight opalescence resulted. After 24 hours a slight, dense precipitate formed. This, however, reacted negatively with phloroglucinol, giving a lemon-yellow color, rather than the amber given by true gums.

The gum-free cheese was allowed to sour spontaneously until putridity began to develop. A 100 gram sample was then tested for gums. The test was negative, showing that the so-called "pseudo gelatin," formed when milk or cream sours, did not affect the result.

The method was tried on the authentic collaborative samples of cheese used by Cross in the preparation of his report on Detection of Gums in Cheese.[1] These samples were described as follows:

No. 1.—High fat; about 0.1 per cent locust bean gum.
2.—Average fat; about 0.05 per cent locust bean gum.
3.—Low fat; no gum, no agar.

The method proposed and also the Cross method gave positive results on Samples 1 and 2, and negative results on Sample 3.

The method presented is capable of detecting as low as 0.05 per cent gums. Gelatin and sour cream protein ("pseudo gelatin") will not interfere with the test.

[1] *Loc. cit.*

TABLE 2.—*Examination of commercial soft curd cheeses known to contain gum*

KIND OF CHEESE	ALCOHOL PRECIPITANT	PHLOROGLUCINOL REACTION	RESULT OF TEST
Cream	Flocculent	Deep Amber	Positive
Cream	Slightly flocculent	Amber	Positive
Creamed Cottage	Flocculent	Deep Amber	Positive
Cream	Flocculent	Deep Amber	Positive
Pot Cheese	Slightly dense	Amber	Positive
Cream	Slightly dense*	Very Light Amber	Doubtful
Cream	Slightly flocculent	Light Amber	Positive
Cream	Flocculent	Deep Cherry Red	Positive

* Precipitate did not form until mixture had stood overnight.

DETECTION OF ADDED GUMS IN ICE CREAM AND ICE CREAM MIXES

The method described for detection of gums in cheese was also applied to vanilla ice cream. The pectin sometimes present in fruit ice creams may interfere. Positive tests were obtained on two samples of authentic ice cream mixes, one containing 0.2 per cent carob bean gum and the other, 0.2 per cent tragacanth.

DETECTION OF AGAR-AGAR IN CANNED CHICKEN

Chicken canned with the addition of agar-agar will be imbedded in a gel containing agar-agar, collagen, soluble proteins, and meat extractives. The method used follows:

Separate from the meat, by means of a thin-bladed spatula, as much of the gel as possible. To 25–50 grams of the separated gel, add 25 cc. of water and warm until the gel dissolves. Filter by suction through a Gooch filter, using a thin mat of coarse asbestos. Adjust the filtrate to pH 2–2.5 (reddish yellow with thymol blue), by the addition of 10 per cent HCl in slight excess and back titration with a normal $NaOH$ solution, add 0.1 gram of pepsin, and digest 1 hour at 38°C. (or allow to stand overnight at room temperature). Add to the digested mixture a weighed amount of trichloracetic acid sufficient to make approximately a 10 per cent solution, warm on the steam bath to coagulate any precipitate formed, cool, and filter. To the filtrate add twice its volume of acetone, mix, and let stand until the precipitate coagulates. From this point, proceed as directed under "Detection of Agar-Agar in Mayonnaise —Separation and Detection of Gums," second paragraph, beginning: "centrifuge the acetone mixture . . . ." Coagulation of the final alcohol precipitate of the agar-agar may be hastened by the addition of a few milligrams of powdered alum.

DETECTION OF SOLUBLE GUMS IN TOMATO PRODUCTS (CATSUP, PURÉE, CONSOMMÉ)

Since tomatoes contain an appreciable quantity of pectin, the separation of gums in a pure state is difficult. Some experience, gained from tests with samples known to be free from gums, is necessary before the analyst can definitely say that gum is present. It is suggested that a

gum-free sample of catsup or purée be run simultaneously with the product under examination for comparison. The method follows:

Transfer 50 grams of the well-mixed sample to an 8 ounce nursing bottle. Dilute to the 5 ounce mark with cold distilled water, shake thoroughly, and let stand for several hours, shaking occasionally. Centrifuge until the sediment is well packed, and decant or siphon 100 cc. of the supernatant liquid. Filter through a Büchner funnel.

Evaporate the filtrate to approximately 30 cc., cool somewhat, add 10 cc. of a 50 g./100 cc. solution of trichloracetic acid, and warm on the steam bath to 70°C. Transfer the mixture to a 50 cc. sediment tube, centrifuge for 10 minutes, and filter. To the clear filtrate add five times its volume of 96 per cent alcohol, and let settle until the precipitate coagulates (hastened by adding a few milligrams of powdered alum). Transfer to a 250 cc. centrifuge (sterilizer) bottle, whirl for 10–15 minutes, decant the supernatant alcohol, wash once with alcohol by decantation, and transfer the precipitate to a folded filter. Dissolve the precipitate in a minimum amount of boiling water (not over 30 cc.). Filter if necessary, using suction; cool below 25°C., and add 3–5 cc. of 10 per cent NaOH solution diluted to 50 cc. with cold water. Let stand in the ice box or under running cold water for 15 minutes. Add 10 cc. of paper pulp suspension, and 10 cc. of HCl (1+2.5) cooled to 20°C. Mix thoroughly and let stand in the ice box for 30 minutes. Filter by suction through a Büchner funnel fitted with a rapid flowing filter paper. Neutralize to phenolphthalein with the NaOH solution, add 1–2 drops of glacial acetic acid, and evaporate to approximately 30 cc. Cool, and add 5 volumes of 96 per cent alcohol. (An immediate precipitate denotes the presence of added gums.) Disregard a slight cloudiness at this point, which is probably caused by small quantities of pectic bodies splitting from the pectic acid precipitate. The conclusion may be confirmed by hydrolysis of the final gum precipitate with dilute acid and subsequent color tests as described previously under mayonnaise. Pectin hydrolyzes to reducing sugars (arabinose and galactose). These sugars, however, do not give a positive resorcinol test until they have stood a half hour.[1]

The presence of gums may be confirmed by determining the alcohol precipitate and pectic acid by A.O.A.C. methods.[2] If the percentage of alcohol precipitate is appreciably more than twice that of the pectic acid, the presence of gums is indicated.

SUMMARY

Methods are outlined for the detection of (1) agar-agar in mayonnaise, (2) gums in soft curd cheeses, (3) gums in ice cream and ice cream mixes, (4) agar-agar in canned chicken, and (5) soluble gums in tomato products.

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[1] Browne, *loc. cit.*
[2] *Methods of Analysis, A.O.A.C.*, 1935, 324.

DETERMINATION OF SPECIFIC GRAVITY*

By E. A. SIEBEL and ARTHUR E. KOTT (E. A. Siebel and Company, Chicago, Ill.)

The specific gravity of a liquid is most accurately determined with a specific gravity bottle or pycnometer. There are many forms of pycnometers, but the usual procedure for filling and weighing them is practically the same for all.

By a modification involving the use of a counterweight, accurate results may be obtained with less manipulation, a quicker calculation, and consequently a shorter total time for each determination.

USUAL PYCNOMETER METHOD

In the customary method, the weights of the empty pycnometer and of the pycnometer filled with water will not be constant, since the weighings are made in air, and are affected by variations in pressure and temperature of the air. These variations produce changes in the weight of the air displaced by the pycnometer, and hence in the apparent weight of the pycnometer, since it is buoyed up while it is being weighed by an amount equal to the weight of the displaced air.

It can be shown that relatively small changes in air temperature and pressure cause variations of several milligrams in the weights of the empty pycnometer and of the pycnometer filled with water. Unless these weights are redetermined whenever there is a change in pressure or temperature, the results obtained may be in error by a significant amount.

In order to provide a definite demonstration of this variation in weight with changing air temperature and pressure, the change in weight that would be expected for a certain pycnometer, as found by calculation from the observed barometric pressure and from the air temperature inside the balance, was compared with the differences actually found between the weights from day to day of the pycnometer filled with water.

The total outside volume of the pycnometer used, which was of the double-wall type, was 130 cc. The volume of the weights was 12 cc. The difference, 118 cc., represents the net volume of air displaced, and variations in the weight of this volume must cause the change in buoyancy effect.

By the use of the equation for the gas law, which gives the relation between the amount of a gas and its pressure, temperature, and volume, it was found that the weight of this 118 cc. of air could be represented as approximately 1.40 P/T, in which P is the pressure in inches of mercury, and T the absolute temperature. From this expression, by considering the pressure and temperature constant in turn at 30 inches and

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, November, 1936.

TABLE 1.—*Comparison of calculated change in weight of pycnometer filled with water with change actually found*

DAY	WEIGHT	TEMPERATURE	MERCURY PRESSURE	CALCULATED CHANGE IN WEIGHT	CHANGE IN WEIGHT FOUND
	grams	*°C.*	*inches*	*mg.*	*mg.*
1	103.1490	27.8	30.25		
2	103.1470	25.5	30.32	−1.5	−2.0
3	103.1510	27.0	30.05	+2.0	+4.0
4	103.1515	26.7	29.76	+1.5	+0.5
5	103.1485	26.1	30.31	−3.0	−3.0
6	103.1515	28.9	30.06	+2.5	+3.0
7	103.1485	27.5	30.76	−4.0	−3.0
8	103.1500	26.9	30.33	+2.0	+1.5
9	103.1510	27.9	30.00	+2.0	+1.0
10	103.1490	25.3	30.16	−2.0	−2.0
11	103.1495	26.0	30.12	+0.5	+0.5
12	103.1515	26.9	29.82	+2.0	+2.0
13	103.1480	25.2	30.27	−3.0	−3.5
14	103.1480	25.0	30.31	0	0

at 300°A, the variation of the weight of the 118 cc. of air with each separately was found.

$$w = 1.4 \times 30/T = 42/T$$
$$w = 1.4\ P/300 = 0.005\ P.$$

From these formulas it was seen that 1°C. increase in air temperature should decrease by 0.5 mg. the weight of displaced air, and thus should increase by 0.5 mg. the weight of the pycnometer filled with water, since if an object is buoyed up less, by a smaller weight of air, its apparent weight is greater.

Similarly an increase of 0.1 inch of mercury in the pressure should increase by 0.5 mg. the weight of displaced air, and decrease by the same amount the weight in air of the pycnometer filled with water.

In Table 1 is given a comparison of the change in weight, calculated from the temperature and pressure, with the change actually observed. The agreement shown proves the dependence of the pycnometer weighings on air temperature and pressure, and indicates the error that may be caused by the use of a water weight determined under different temperature and pressure from those obtaining at the time of the weighing of the pycnometer filled with the liquid of which the specific gravity is being determined.

There was a range of 4.5 mg. in these weights of the pycnometer filled with water. They were taken at about the same time each day, and the air temperature was fairly uniform. If some of the weighings had been made early in the morning, when the air temperature was 21° or 22° C., a total variation of about 7 mg. would have been found. An error of

corresponding size in an alcohol determination in beer, for example, would amount to 0.00014 in the specific gravity, as the pycnometer was of the 50 gram size, and this amount would constitute an error of 0.10 per cent in alcohol by volume, due solely to failure to take account of the variable buoyancy effect.

The relative humidity was observed at the time these weighings were made, but was found to affect the weight of displaced air by only a few tenths of a milligram. Errors caused by the humidity are of another sort, involving condensation of moisture on the pycnometer during the weighing; they will be considered later.

A single wall 50 gram pycnometer, with a smaller outside volume, may be subject to only about half the error found possible in the case of a pycnometer with an outside volume of over 100 cc. Yet the error will still be considerable enough, in comparison with the accuracy of filling and weighing, to make a correction desirable.

If a counterweight of about the same outside volume as the pycnometer is used for the weighings, there will be no variable buoyancy effect, and the error will be eliminated.

A counterweight can of course be used with any type of pycnometer, to avoid the error that is introduced by changes in buoyancy effect if the weight of the pycnometer filled with water is not determined often enough, or to avoid the necessity for frequent determinations of this weight. The pycnometer used may be of the Reischauer type, which is filled through a long narrow neck, and the liquid level adjusted to a mark after the pycnometer has been kept a certain time in a water bath at 20°C. Or it may be of the type that has a removable ground-glass plug with a capillary bore, which is filled to the top of the plug. This type may have a single or double wall; if a counterweight is used, the advantage the single wall type possesses of smaller outside diameter is eliminated, and the slower heat transfer through the double wall makes that type more desirable. Either of these kinds, with removable plug, may be used with or without a ground-glass cap to fit over the plug.

NEW METHOD

The new method described was developed with the particular purpose of making possible a large number of accurate specific gravity determinations in a short time. The apparatus required consists of pycnometer, counterweight, beaker, and thermometer. The pycnometer is of the double-wall type; a ground plug with capillary bore fits in the neck; the top of the plug is polished. The cap is not used. The plug may be ground further so that the weight of contained water at 20°C. is exactly 50 grams; this simplifies the calculation, but it is not necessary, since a difference from 50 grams up to 0.2 gram may be accurately corrected for. The counterweight is made of glass and sealed. If provided with a

small hook at the top, it may be suspended from the beam of the balance, out of the way of weights to be placed on the pan below. It is adjusted to have a weight about 5 grams less than that of the pycnometer filled with water, and an outside volume within 5 cc. of the outside volume of the pycnometer. A small aluminum beaker (or glass for acids and alkalies), and a thermometer correct to 0.1° at 20°, are also used.

The empty weight of the pycnometer is required only once, to determine whether a correction is necessary in the calculation. The pycnometer is cleaned with dichromate-sulfuric acid solution; rinsed with water, alcohol, and ether; and dried by having a current of air drawn through it. The plug is cleaned and dried in the same way, and put in place. The outside of the pycnometer is then wiped thoroughly with a dry towel, and it is weighed on an analytical balance.

Next the weight in air of the pycnometer filled with distilled water at 20° is determined. This weight also is required only once, although it may be advisable to check it occasionally to make sure there has been no change in the weight of the pycnometer itself (by the chipping off of a small piece of glass during handling, for example). The pycnometer itself is first cooled down, provided the room temperature is above 20°, by immersing it for a short time in a bath of ice and water, and then rinsed out several times with distilled water at a temperature between 19° and 20° to cool down the inside bulb. The water is contained in the beaker, and stirred with the thermometer. Then the pycnometer is rinsed out three times with distilled water at about 19.9°, and when the water in the beaker has come to exactly 20° the pycnometer is rinsed quickly once more and then filled as rapidly as possible. After a wait of two or three seconds to allow any air bubbles to rise to the top, the plug is put in place firmly, and the top of it wiped off. The pycnometer is then placed in the ice and water bath, being submerged to the plug, but without allowing any water to get on the top surface, where it might flow into the capillary. When the level in the capillary has fallen about 1/16 of an inch, the pycnometer is removed and wiped off roughly with one towel and then carefully with another dry towel, and weighed, the final adjustment of the weights being made just as the liquid level rises to the top of the capillary. The pycnometer is then emptied, filled again with distilled water at 20° in the same way, and weighed again. These two weighings should check to within 0.5 mg.

The pycnometer is filled with the liquid of which the specific gravity is being determined in exactly the same manner as just described for water, the liquid being brought to exactly 20° and then poured quickly into the pycnometer, which has also been brought to 20° by several rinsings. When the liquid level has fallen slightly in the capillary the pycnometer is removed from the ice-water bath, wiped dry with a towel, and weighed, as before.

CALCULATION

Since the counterweight weighs more than the empty pycnometer, weights must be placed on the same pan of the balance as the pycnometer when it is weighed empty. Then when the pycnometer has been filled with distilled water at 20°, a certain weight in addition to the counterweight on the other pan will be required to balance it.

As an example, suppose these weights are 44.5032 and 5.3076 grams. Since the weights were on different sides of the balance the sum, 49.8108 grams, is the weight of contained water at 20°.

If the weight of the pycnometer filled with the liquid were 6.9244, the specific gravity at 20°/20° would be—

$$\frac{49.8108+(6.9244-5.3076)}{49.8108}=\frac{49.8108+1.6168}{49.8108}=1.03246.$$

This illustrates the general method of figuring the result; the difference in weight from water is added to the weight of contained water (for liquid heavier than water) and the result is divided by the weight of contained water. If the pycnometer should hold exactly 50 grams the calculation is simpler. In other cases it can be simplified by applying a correction for the difference from 50 grams in the weight of contained water by making the denominator 50.

With the pycnometer in this example, $50.0000-49.8108=0.1892$. Now if the weight of contained water is assumed to be 0.1892 grams greater, then the numerator will have to be increased slightly more than 0.1892, because the liquid is heavier than water.

For approximately 50 parts of water, it was found by the weighing that the liquid was approximately $1.6168(6.9244-5.3076)$ parts heavier. Therefore 1.617/50 is the ratio of the excess weight of the liquid to the weight of water. (The more exact figures do not increase the accuracy for this purpose.) By applying this ratio to the increase it is desired to make in the weight of water, 0.1892 gram, the amount in addition to this to be added to the weight of the liquid will be—

$$1.617/50\times0.1892=0.0060 \text{ grams.}$$

The revised calculation is made therefore as follows:

$$\frac{49.8108+1.6168+(0.1892+0.0060)}{49.8108+0.1892}=\frac{50.0000+0.0060+1.6168}{50.0000}$$

$$=\frac{51.6228}{50.0000}=1.03246.$$

The amount 0.0060 is called the correction, and 50.0060 is the figure to which the difference in weight between the pycnometer filled with the liquid and with water has to be added, in order that the sum may be divided by 50.

It may appear that the correction would have to be changed for each determination, but in practice this is not the case. Usually the specific

gravities of a considerable number of liquids nearly the same are required, and the same correction may be used. For example, in the case of the approximately 8 per cent laboratory worts of which the specific gravity is determined in the analysis of malt, the error caused by using an average correction of 0.0060 (for the pycnometer described in these examples) would be less than 1 in the 5th decimal place of the specific gravity. For a pycnometer with a contained water weight closer to 50 grams, it would be even less.

If the specific gravity of a liquid lighter than water is to be determined, the correction must be applied in the other direction. For example, suppose the same pcynometer filled with an alcohol-water mixture weighs 4.9470 grams. The difference from water is $5.3076-4.9470=0.3606$, and this figure is to be subtracted rather than added. Figuring as before, the correction (also to be subtracted) is

$$\frac{0.361}{50}\times 0.1892=0.0014.$$

The calculation is

$$\frac{49.9986-0.3606}{50}=0.99276.$$

In the determination of specific gravities of alcohol distillates in the analysis of beer, as with the malt worts, the error caused by using an average correction is negligible.

Even if the specific gravity of the liquid is quite different from that of water, the same method of calculation gives accurate results. The only data required in each determination are the known values for the pycnometer, and the one weight, that of the pycnometer filled at 20° with the liquid. The following two cases illustrate the method of calculation. With the pycnometer described (water weight, 5.3076; difference of contained water from $50=0.1892$):

1. For an approximate 50% alcohol solution:
   weight 1.9132;

$$5.3076-1.9132=3.4944;\quad \frac{3.494}{50}\times 0.1892=0.0132;\quad \frac{49.9868-3.4944}{50}=0.92985.$$

2. For sulfuric acid of about 50° Baumé:
   weight 31.6550;

$$31.6550-5.3076=26.3474;\quad \frac{26.347}{50}\times 0.1892=0.0996;\quad \frac{50.0996+26.3474}{50}=1.52894$$

DISCUSSION

A useful comparison may be made between the procedure described and the procedure specified by many authorities, according to which the pycnometer is filled and placed in a water bath, the level of the liquid

adjusted to a mark on the pycnometer at 20°C. after it has been kept in the bath a while, the pycnometer removed and dried, allowed to stand until it comes to room temperature, and then weighed, and the same procedure followed with water.

The weighing of the pycnometer after it has come to room temperature is evidently intended primarily to eliminate condensation of moisture on it. By the method presented there is a possibility of condensation, due to the fact that the pycnometer is chilled in ice and water after being filled. However, its outside surface is warmed by wiping with a towel, before it is weighed, and this warming raises the temperature of the outside glass several degrees above 20°. The lowering of temperature by chilling in the ice and water bath is necessary in order that the weighing may be done before the liquid comes out of the capillary. But then for the liquid to rise almost to the top of the capillary, the inner bulb has to be brought back to 20° by the heating effect of the wiping of the outside glass and the contact with room air. It is clear that for the inner bulb to be heated to 20° again, through the space between the walls, the outer glass must reach a temperature considerably above 20°.

It has been found in practice that in the time needed to made the weighing there is no increase in the weight of the pycnometer, such as would be caused by condensation of moisture.

Less apparatus is required for the new procedure. No 20° water bath is necessary, and only the one pycnometer and counterweight. If many determinations are to be made, the other method for practical use requires several pycnometers, because if only one were used, each determination would take more than half an hour. With the pycnometer and procedure here described, each determination takes about 5 minutes, including the calculation.

Because of the removable plug, the pycnometer can be filled and emptied quickly and easily. The slower filling of the other type (such as the Reischauer pycnometer, which must be filled through a funnel because of its long narrow neck) results in considerable error by vaporization in the case of solutions of volatile liquids, such as alcohol-water mixtures high in alcohol.

SUMMARY

The weight of a specific gravity pycnometer varies with the air temperature and pressure because of change in the air buoyancy effect.

For this reason, to give more accurate results, the standard method for the determination of specific gravity requires frequent weighings of the empty pycnometer and of the pycnometer filled with water.

If a counterweight is employed, changes in the air buoyancy effect are eliminated and the necessity for these frequent weighings is removed. Only one weighing is then needed for each determination.

By the application of an accurate correction for the difference in the weight of contained water from 50 grams the calculation of the final result can be simplified. Instead of two or three subtractions and a long division involving numbers with 5 or 6 digits, only a subtraction or addition and a division by 50 are required.

For the rapid determination of a large number of specific gravities the new method therefore has the advantage of greater speed with no sacrifice of accuracy.

BOOK REVIEWS

Distillation. By J. Reilly, M. A., D. Sc., with a Foreword by Professor S. Young, F.R.S. Pp. VIII and 118. London, Metheren and Company, Ltd., 1936. The Chemical Publishing Company of N. Y., Inc., 148 Lafayette St., New York, N. Y. Price. $1.25.

In this little brochure of 118 pages (4"×6"), Professor Reilly has skillfully condensed an immense amount of material on distillation. The introduction covers a mathematical treatment of distillation, vapor pressure, latent heat, and liquid-vapor equilibrium curves. Discussion of fractional distillation includes a detailed analysis of the McCabe-Thiele graphical method for calculating the number of theoretical plates in a packed or plate type column. In a few pages, the author summarizes developments in the design and construction of both laboratory and plant fractionating columns, and virtually no contribution of importance along this line has escaped his careful combing of the literature.

A somewhat unusual feature of the book is the extent to which Professor Reilly goes into accessory apparatus. This includes devices for the production and measurement of low pressure, for the automatic control of pressure, and for the control of reflux, as well as a description of the automanometer as an adjunct for accurate low pressure measurement and of the various other parts that go to make up a complete distillation assembly. On the other hand, comparatively little attention is given to column insulation and means for the attainment of adiabatic conditions, a matter of considerable importance in laboratory columns. This subject has been fully treated in Podbielniak's publications, to which the author refers in another connection.

The reader will find the chapter on vacuum fractionation particularly informative and useful. The discussion on the distinction between ordinary distillation involving "active ebulition" and that of "evaporative distillation without ebulition," together with illustrations of practical apparatus, contributes much to a clearer understanding of the fundamentals of high vacuum technique.

The expert and concise manner in which the troublesome subject of azeotropic mixtures is treated by Professor Reilly deserves particular mention. The theoretical and practical features are discussed, and special attention is given to the use of the formation of other azeotropic mixtures by the addition of a third component in alcohol distillation and dehydration. The chapters on sublimination and destructive distillation are considered somewhat briefly. Description of laboratory technique, and citations on the subjects will serve as a useful guide for more detailed study.

There are a few obvious typographical omissions, such as the absence of the diagram of the McLeod gauge on page 41, that of the distillation retort on page 105, and the statement that "santolol boils at 30.1°C." These will doubtless be corrected in the next priting.

It is well, perhaps, to point out to the student that Professor Reilly's little monograph is not an elementary book. Preliminary study of such texts as Young's, "Distillation Principles," Robinson's, "Elements of Fractional Distillation," and others will be found very helpful. For its survey of the literature, Professor Reilly's book will also serve as a particularly valuable guide.—S. Palkin.

Humus, Origin, Chemical Composition and Importance in Nature. By Selman A. Waksman. 494 pp., 45 figures. The Williams & Wilkins Company, Baltimore, 1936. Price $6.50.

Professor Waksman is well qualified to write on the subject of humus. Some idea of the amount of work he has done in this field can be gained from the fact that the

bibliography of this publication contains 26 entries under his name. The book is divided into three main parts, the first of which (88 pages) deals with the "Historical Development of our Knowledge of the Chemical Nature of Humus, Its Formation, and Its Role in Plant Nutrition." The second, and by far the largest part (237 pages) discusses the "Origin and Nature of Humus." The third section of 81 pages deals with the "Decomposition of Humus, Its Functions and Applications."

The compilation of this work must have been a stupendous undertaking. The bibliography contains 1371 entries. all of which are referred to in the text. The earliest reference noted by the reviewer was to H. Boerhaave's "A New Method in Chemistry" (1727). Some earlier writings, *e.g.*, Theophrastus, 373–328 B. C., are referred to in the historical discussion but do not form part of the bibliography. Several of the references are to papers published as late as 1935. Evidently Professor Waksman has covered the literature in a thorough and complete manner.

The book is well printed, but except for glazed paper necessary to reproduce the four photographs ordinary unglazed paper is used. Most of the charts appear to have been lettered with a typewriter and since the amount of reduction used varied considerably, the lettering on some cuts is painfully fine and on others is so large as to be out of proportion. Cross lines are put in on some charts and left out of others, although the scales may be closely numbered. These charts are serviceable but are certainly not attractive.

In an appendix of 13 pages the various methods of estimating the humus content of soils and of analyzing the humus itself are discussed briefly. The impression one gets from reading this section is that the best of the available methods leaves much to be desired. Some 20 pages of Chapter IV are also devoted to humus determination.

Professor Waksman has placed interesting little quotations at the heads of many of his chapters. Not a few of these are from the writing of such well-known soil scientists as Marbut, Van Bemmelen, Schreiner, and Shorey. This is a practice that might well be more widely adopted.

All workers in this and related fields will be grateful to Professor Waksman for having brought together and correlated the tremendous mass of confused information and misinformation that exists on the subject of humus. The work is a mine of information, and it is difficult to see how any worker in soil science or related subjects can afford to be without it.—COLIN W. WHITTAKER.

Perry Fox Trowbridge, 1866–1937

PERRY FOX TROWBRIDGE

One day in the early summer of the year 1907, Dr. Perry Fox Trowbridge came bustling into the room where I was analyzing boiler waters for Professor S. W. Parr and said to me: "I hear you have decided to give up your fellowship here in order to find some employment that will fully finance the graduate work you wish to take. Before deciding to accept any other offer I want a chance to tell you about the opportunities at the University of Missouri where I am going to build up a department of agricultural chemistry." Anyone who knew Dr. Trowbridge need not be told that he convinced me of the desirability of the work he offered me. Thus began an intimate association that continued until the day of his death.

Being in need of some pocket money, I had previously worked for a number of hours in Dr. Grindley's laboratory helping to make water extracts of beef muscle and to prepare samples of the different groups of proteins that could be prepared in such manner from beef flesh. The work required an all-night vigil on the part of the men, who were also on duty the following day. It was a tiresome task that required some manual skill, but the task was made interesting and instruction in the requisite skill was imparted by the vigorous, stocky, gray-haired Trowbridge who had come to the University of Illinois at the age of forty to build a new career for himself.

This was not the first time that Perry Trowbridge had had to take off his coat, roll up his sleeves, and work hard to accomplish the thing that had to be done. As a youth in a family of nine children with an invalid country doctor for a father, he began his struggle for an education. After some experience as a teacher he entered the Michigan State Normal College and obtained the degree of Bachelor of Pedagogy in 1889. Then followed three years of teaching in Kalamazoo College, one year at the Michigan State Normal College, and eight years at the University of Michigan. One year of this latter time was spent in graduate study at the German Sugar Technology School and the University of Marburg. In 1902 the beet sugar industry of Michigan and California attracted him, but after two years' work in this field had convinced him that it did not hold sufficient promise for the future, he returned to college at the University of Illinois, where he worked for his doctorate (Ph.D., 1906) and taught as a member of the chemistry faculty His graduate work here in the field of the chemistry of meat led to his being taken in the summer of 1907 to the University of Missouri to build up the Department of Agricultural Chemistry and to have charge of the chemical work connected with an extensive project dealing with the nutrition of meat animals on which Dean H. J. Waters was spending a lion's share of the research funds of the Agricultural Experiment Station.

For the next five years I lived in his home and so I had an opportunity of seeing him both as a boss and as a host to a paying guest As a chemist he was exacting in his requirements for accuracy and honesty, as a teacher he was inspiring, and as a landlord he was a cheerful friend who did not hesitate to stay up most of the night to help nurse me when I had a severe attack of food poisoning. There was no task connected with the nutrition work with beef steers and hogs which he could not and did not perform. He never asked a chemist to undertake any disagreeable task which he himself would not carry out. He loved a good story and enjoyed a joke on one of his chemists or on himself with

equal amiability. He was fond of a game of chess, which he proceeded to teach me. I never will forget the gleam of joy that came into his eye when he checkmated me, as he usually did, and that look of surprise which still could not destroy the gleam when, on rare occasion, I succeeded in checkmating his king.

As chief chemist of the Missouri Agricultural Experiment Station and State Chemist, he became associated with the work of the Association of Official Agricultural Chemists in 1907. He was very active as an associate referee, referee, chairman or member of its committee on the revision of methods, vice president and president (1918 and 1919). When he left Missouri in 1918 to become director of the North Dakota Agricultural Experiment Station, he gradually gave up his active connection with the Association, for he believed firmly that the chemists at the station should have their opportunities of contact with this group of fellow chemists. His new duties caused him to be active in the work of the Association of Land Grant Colleges, where he served as chairman of the committee dealing with Purnell Fund projects.

In 1924 I was helping the National Live Stock and Meat Board to get under way its project dealing with factors affecting the quality and palatability of meat. At this time a committee was formed with Dean F. B. Mumford as chairman. In 1926 Dr. Trowbridge was added to the committee. He took over the duties of chairman, and later this work became one of the accepted projects for the expenditure of Purnell funds. This was distinctly a cooperative project involving the working together of over two dozen agricultural experiment stations, the Bureau of Animal Industry and the Bureau of Home Economics of the U. S. Department of Agriculture, the staff of the Meat Board, the Institute of American Meat Packers, and a number of individual meat packing plants. Dr. Trowbridge's knowledge, wide experience, and good spirit contributed much to the success of this work.

Dr. Trowbridge had no bad habits so far as I knew. He was an active worker in the Baptist Church and in the Y.M.C.A. He did not let my apparent lack of interest in such things interfere with our friendship nor did he try to "preach" to me. However, on one occasion of promotion which came my way, he suggested to me that one of the requisites for success in any community was an active interest in the life of that community including its religious activities. A number of years later when a change of employment brought me to a city where there was a church of the kind to which I belonged, I took his advice to heart. I should have liked to have him know this, but I think he never did.

Perry Fox Trowbridge was born in Three Rivers, Michigan, April 25, 1866, and passed away after a few months' illness due to a bad heart on May 15, 1937, at his home in Fargo, North Dakota. A formal record of some of his achievements can be found in Rus, American Men of Science, and Who's Who in America. An account of him as a contemporary American chemist, written by a former student and colleague, Dr. L. D. Haigh, was published in the News Edition of Industrial and Engineering Chemistry, Volume 23 (1931), page 968. A necrology appears in the June 20, 1937 issue of the News Edition of that journal. Very shortly before his death his portrait was painted for hanging in the galleries of The Saddle and Sirloin Club in Chicago, a tribute that was barely completed before his death ended an active career which had been crowned with success greater than that which is the lot of less worthy and lovable characters.

C. Robert Moulton

SECOND DAY

TUESDAY—MORNING AND AFTERNOON SESSIONS—Continued

REPORT ON DRUGS

By L. E. WARREN (U. S. Food and Drug Administration Washington, D. C.), *Referee*

Since the last meeting of the Association, three events of considerable importance to drug analysts have occurred. These comprise the publication, respectively, of the United States Pharmacopoeia XI, the National Formulary VI, and the fourth edition of *Methods of Analysis*, A.O.A.C. The last-named of these is the product of the labors of this Association, and the drug chapter in the book is the outcome of researches in this section. Since this section is responsible for this work, a brief review of it is in order at this time.

METHODS OF ANALYSIS

Exclusive of microchemical methods, but including biological methods, the drug chapter in *Methods of Analysis* describes one or more methods for the assay of about 100 drugs or mixtures of drugs. In addition, microchemical methods are given for the detection of 26 alkaloids and 12 synthetic substances. The previous edition contained directions for the detection of 14 alkaloids by microchemical tests, but no microchemical tests for synthetics were included.

Since the 1930 edition appeared certain drugs have attained greater prominence in medicine. Methods for their evaluation have been introduced. Among these may be mentioned calcium gluconate, carbon tetrachloride, psyllium seed, tetrachlorethylene, and theobromine in theobromine calcium. Among other drugs for which assays have been introduced are the following: Stramonium ointment, belladonna ointment, elixir of terpin hydrate, hypophosphites, improved assays for santonica, iodoform gauze, ipomea, jalap, mercurochrome, nitrites in tablets, podophyllum, sulfonal, and trional.

Methods for the analysis of several preparations were deleted because the U.S.P. XI had provided assays for them. Others were deleted because they had been incorporated in the N.F. VI.

In addition to the chapter on drugs, the book gives some methods in other chapters that are of much interest to drug analysts. Examples: Latest and most approved methods for the preparation of aldehyde-free alcohol and of standard alcoholic potassium hydroxide solutions may be found in the chapter on Oils, Fats, and Waxes; a method for the determination of volatile oils in spices, applicable to drug products such as clove, cinnamon, and nutmeg, is given in the chapter on Spices (p. 447). It

might also be applied to chenopodium, lemon peel, lavender, peppermint, sassafras, etc.

The Association now has a Referee on the Preparation and Standardization of Volumetric Solutions. Methods for the standardization of sodium hydroxide and hydrochloric acid have already been adopted and are included in the appendix of the book.

Microchemical Methods for Alkaloids.—Previous to this year the Association had adopted microchemical methods for the identification of 26 alkaloids. Only a few alkaloids that are used in medicine remain to be studied. This year anabasine, apomorphine, hydrastine, and pelletierine were studied by the direction of the Association. In addition to the microchemical test for theophylline already adopted by the Association, further studies were made, mercuric chloride being used as reagent. Satisfactory tests were developed for apomorphine, hydrastine and theophylline. A new test for apomorphine was observed by Analyst Shupe. Preliminary tests on pelletierine were not satisfactory as the commercial product is a mixture of alkaloids, and no characteristic microchemical tests for anabasine were obtained. All the recommendations of the associate referee are approved.

Microchemical Methods for Synthetics.—Previous to this year the Association had adopted microchemical methods for the identification of 12 synthetic substances, but the subject of synthetics has really just been begun. This year microchemical methods for barbital, phenobarbital, amytal, and ethylhydrocupreine were investigated. The recommendation of the Associate Referee that these methods be adopted as tentative is approved. It is also recommended that study next year be confined to acetylsalicylic acid, benzoic acid, salicyclic acid, and diallylbarbituric acid.

Hypophosphites.—This topic has been studied for five years. Two years ago a method for total hypophosphites in absence of phosphates was adopted as tentative. This year the Associate Referee was assigned the study of methods for the determination of hypophosphites in the presence of phosphates. During the year the Bruening volumetric method for the determination of hypophosphites in sirup was developed by one of the members of the Association.[1] This method depends on the oxidation of the hypophosphite to phosphate by bromine liberated from a standardized solution of bromide-bromate. The method is said to be accurate and rapid. Some collaborative work was carried out by the Associate Referee and his aides. The time allowed for the reaction to take place appears to be an important consideration. The Associate Referee's recommendation is approved.

Santonin, Phenolphthalein, and Calomel in Tablets.—The subject santonin (which includes santonica) has been studied by various associate

[1] *J. Am. Pharm. Assoc.*, 25, 19, 1936.

referees over a period of years. Methods for the assay of santonin in santonica, and in several pharmaceutical preparations have been adopted by the Association. Also the Association has adopted methods for the determination of santonin, phenolphthalein, and calomel separately. This year the associate referee developed a method for the assay of each ingredient. In the hands of collaborators this method gave excellent results for santonin and calomel but erratic values for phenolphthalein. The recommendation of the Associate Referee that the subject be studied further is approved.

Benzyl Compounds.—This subject has been under consideration for five years. The earlier work resulted in the development of methods for determining benzylic alcohol in water and salt solutions and in mixtures with ethyl alcohol. This year the Associate Referee studied benzyl benzoate, particularly in solutions with olive oil. A method evolved includes saponification of the product and separation of the benzoic acid from the other acids by its solubility in water. The benzoic acid in the aqueous solution is eventually titrated. If the diluent is known to be olive oil, the benzyl benzoate may be calculated from the refractive index of the mixture. The Associate Referee's recommendations are approved.

Rhubarb and Rhaponticum.—This topic has been under consideration for a number of years. Some interesting reports have been received, but these have not resulted in the adoption of any methods for distinguishing between the two drugs.

This year the Associate Referee developed a method by which he compares the activity of a water-soluble cathartic with elaterin β, using *Daphnia magna* as the test animal. He recommends that the topic be continued in order that collaborative trials may be carried out. The Referee concurs.

Hexylresorcinol.—This subject has been under consideration for several years. A bromination method was developed, but it is not completely successful. This year the topic was reassigned. The Associate Referee modified the bromination method previously studied. The improved method gave good results in the hands of collaborators. The Associate Referee's recommendations are approved.

Ergot Alkaloids.—This topic has been under consideration for five years. A colorimetric method for the determination of the two ergot alkaloids, ergotamine and ergotoxine, has been adopted, *Methods of Analysis, A.O.A.C.*, 1935, 583. During the past year work was undertaken to develop a method for the identification of the newly-discovered alkaloid ergometrine. Methods for the separation of ergometrine and ergotoxine were employed. The Associate Referee recommends that further work be done. The Referee concurs.

Nitroglycerin in Mixtures.—The Associate Referee has done a great deal of analytical work on this subject this year. He applied the double

distillation method, *This Journal*, **15**, 140 (1932), to the problem. He has employed various mixtures. The results in most cases are promising. With reduced iron the results are high. No collaborative work was carried out. The Associate Referee's recommendation that the subject be continued is approved.

Guaiacol.—This difficult topic has been under consideration by the Association for six years, but no substantial progress has been made. At the last meeting a new associate referee was appointed. However, he was unable to do any work during the year. It is recommended that the subject be continued.

Physiological Testing.—The Associate Referee reported that no work was done and that it was impossible at present to give a schedule of contemplated activities. It is recommended that the subject be continued.

Iodine Ointment.—Last year a method for the determination of total iodine in iodine ointment was recommended by the Associate Referee for adoption by the Association. In view of the fact that the method had been accepted for inclusion in the U.S.P. XI, the method was not adopted by the Association (to avoid duplication). This year methods were studied for determining combined and uncombined iodine in the ointment and some attention was given to the organically combined iodine. Three methods for free iodine were tried. None of the methods was sent to collaborators. The Associate Referee recommends, and the Referee concurs, that certain of the methods already tried by the Associate Referee be subjected to collaborative study.

Acetphenetidin in presence of caffeine and aspirin.—The Associate Referee continued to experiment with the published method, *This Journal*, **19**, 520, 1936. His findings were satisfactory for acetphenetidin and aspirin but were high in general for caffeine (100–122 per cent). The Associate Referee recommends that the topic be continued in an effort to improve the method of assay for caffeine. The Referee concurs.

Pyridium.—The assay of pyridium has been under consideration for several years, *This Journal*, **17**, 53, 421 (1934); **18**, 514, 551 (1935). A microchemical, qualitative test for pyridium was adopted by the Association in 1932, *Ibid.*, **16**, 54 (1933). Two years ago the Associate Referee proposed a chemical assay that depends on the extraction of the base with chloroform from an alkaline solution with subsequent evaporations of the solvent. This was not adopted because of the volatility of the base. Last year another Associate Referee was appointed. This year the Associate Referee tried reduction with a standardized solution of titanous chloride. The results were not entirely satisfactory, consequently no collaborative work was done.

Aminophylline.—This is a new topic. Aminophylline is a combination of theophylline and ethylene diamine. Since the Association had adopted no method for the assay of theophylline the Associate Referee first sought

a satisfactory assay process for this alkaloid from the literature. After preliminary trials of several published methods, two procedures were selected for collaborative study. These were applied to various preparations of theophylline including aminophylline. One of the methods gave fairly good results. The results by the other were erratic. The recommendations of the Associate Referee are approved.

Gums in Drugs.—Methods for the identification of gums have undergone scrutiny for two years, but none has been adopted. Methods for single gums were moderately satisfactory but they were found inadequate for mixtures. Also the reactions observed for pure gums did not respond with the gums after separation from pharmaceuticals. This year the Associate Referee precipitated certain gums from mixtures by alcohol and subjected the precipitate to microscopic examination. The results with Irish moss, quince seed, and tragacanth were promising but no collaborative work was done. The Associate Referee's recommendation that the subject be continued is approved.

Cinchophen in Sodium Bicarbonate.—Some tablets of cinchophen contain sodium bicarbonate to render the drug more soluble. The presence of this alkali complicates the analysis by the usual methods. The National Formulary assay method does not provide for this contingency. Accordingly, an Associate Referee was appointed to study the topic. A method was developed by which the cinchophen is extracted with a solvent and subsequently titrated. The results obtained by collaborators are excellent. The Associate Referee's recommendations are approved. The Referee suggests that the mixtures of cinchophen with salicylates be studied.

Stability of Potassium Iodate Volumetric Solution.—This subject has been under consideration for two years. Last year the Associate Referee reported that a 0.025 *M* standardized solution of potassium iodate under collaborative study remained stable during 9 months' storage. The same solution has now been observed over an additional period of 11 months. The results show that potassium iodate solution (0.025 *M*) is stable over such periods as the solution is likely to be kept.

Since standard solutions of potassium iodate of various molarities are described in U.S.P. XI, the N.F. VI, and in *Methods of Analysis, A.O.A.C.* 1935, the Referee believes that there is but little necessity for additional standard solutions of this reagent. He does not, therefore, concur in the first two of the Associate Referee's recommendations. He approves of discontinuing the topic.

Dinitrophenol.—This topic has been under consideration for two years. The Associate Referee has studied the bromination method and has determined the limits of acidity, concentration, and time necessary to yield the best results. The collaborative findings are excellent. The recommendation of the Associate Referee that the method be adopted as tentative and that the subject be closed is approved.

Theobromine in Theobromine Calcium Tablets.—No report was received. It is recommended that the subject be continued.

Chlorbutanol.—No work was done this year. It is recommended that the topic be continued.

Aspirin and Phenolphthalein Mixtures.—This subject has been under consideration for two years. This year the Associate Referee applied the Hitchens method to a mixture of phenolphthalein, acetylsalicylic acid, and starch. His results were promising but the findings by the collaborators were disappointing. The recommendation of the Associate Referee that the subject be continued is approved.

Homatropine in Tablets.—This is a new topic. A microchemical identification test for homatropine had been adopted by the Association but no quantitative methods had been studied. The Associate Referee applied well-known analytical procedures to the problem with good results. Owing to lack of time, no collaborative work was done. The recommendation of the Associate Referee that the topic be continued is approved.

Cubeb.—This new topic was assigned after the last meeting. At that time the National Formulary had not adopted a method for the assay of cubeb. The A.O.A.C. has adopted tentatively a general method for the determination of volatile oils in spices (*Methods of Analysis, A.O.A.C.*, 1935, 447). This year the Associate Referee adapted the A.O.A.C. method to the assay of cubeb. The results of the collaborative study are in reasonable agreement, except in respect to the ester number of the volatile oil. The recommendation of the Associate Referee that his method for the assay of cubeb be adopted as tentative is not approved by the Referee, since he believes that more extensive collaborative studies should be made. The second recommendation of the Associate Referee that further work be done on the topic is approved.

Methods for the Titration of Alkaloids.—This subject was taken up after the last meeting. Examination of the Drug Chapter in *Methods of Analysis* revealed a lack of uniformity in the directions for the titration of alkaloids. It seemed desirable, therefore, to make a study of all the titration procedures for alkaloids described in *Methods of Analysis.* Accordingly, an Associate Referee was appointed. His work has consisted chiefly in purifying commercial specimens of alkaloids and in determining the purity of the resultant products. Also he has made a considerable study of the literature. He recommends that the subject be continued. This is approved. He also recommends that collaborative studies be made on the purification of alkaloids by double extraction methods. The Referee believes that this topic lies outside the subject as originally assigned and does not approve the recommendation at this time.

Assay of Emulsions.—The U.S.P. XI and the N.F. VI describe several emulsions, some of which are of great importance in therapy. However,

neither compendium gives any methods for the analysis of such preparations. The A.O.A.C. has adopted methods for the analysis of mineral oil—soap emulsions and for the determination of fat in milk, but these methods have not been adopted for medicinal emulsions. The Referee recommends that the study of emulsions be undertaken with special reference to the emulsions of cod liver oil and liquid petrolatum.

Elixir of Terpin Hydrate and Codeine.—A method for the determination of terpin hydrate in elixir of terpin hydrate has been adopted by the Association. Codeine is frequently present. No method for the determination of this alkaloid in presence of terpin hydrate has been adopted. It is recommended that the determination of codeine in elixir of terpin hydrate and codeine be studied.

Ointment of Mercuric Nitrate.—The N.F. VI describes the preparations of ointment of mercuric nitrate but provides no method for the assay of the product. It is recommended than an associate referee be appointed to study the subject.

Phenobarbital and Aminopyrine Mixtures.—Preparations are marketed containing aminopyrine and phenobarbital. Satisfactory methods for the separation of these drugs have not been published. It is recommended that this subject be studied.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS

By C. K. Glycart (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In conformity with the recommendation made last year that attention be given to apomorphine, hydrastine, pelletierine, and anabasine, the work on this subject was continued. In the preliminary study with the various reagents, potassium iodide and apomorphine hydrochloride formed brilliant crystals, which have, according to Stephenson,[1] sharp clear-cut angles like those of a diamond. The test used for hydrastine is essentially as devised by H. I. Cole.[2] The crystals with potassium ferrocyanide reagent are produced after the alkaloidal solution is acidified with hydrochloric acid. The reaction is described as an addition type and the formula of the compound is $B_2H_4Fe(CN)_6$ X H_2O.

With regard to pelletierine and anabasine, further work is necessary before these alkaloids can be submitted to collaborative study. As stated in the report last year, with Kraut's reagent caffeine, theobromine, and theophylline in 1:1000 solution form crystals similar in appearance. For this reason theophylline was resubmitted with directions for a confirmatory test by mercuric chloride reagent, which forms spheres and

[1] Microchemical Tests for Alkaloids, p 20 (1921).
[2] *Philippine J. Sci*, 23, 97-101 (1923).

double tufts of dense radiating needles, differing from the long radiating needles of caffeine with the same reagent.

Directions for the tests, control specimens consisting of apomorphine hydrochloride U.S.P., hydrastine hydrochloride N.F. VI, theophylline U.S.P., and samples labeled No. 1, 2, and 3 for identification were sent to the collaborators. Sample No. 1 consisted of apomorphine hydrochloride, No. 2 of a 1:100 solution of hydrastine hydrochloride, and No. 3 of a 1:150 solution of theophylline. The material for controls was tested and considered sufficiently pure for the work. The directions were published in *This Journal*, **20**, 79 (1937).

RESULTS AND COMMENTS

Chemical Control Laboratories, Eli Lilly and Company.—No. 1, apomorphine; No. 2, hydrastine; No. 3, theophylline. We found no particular difficulty in applying these tests. In the test for apomorphine hydrochloride the crystals formed quite slowly although they appeared to be sufficiently distinct.

Jonas Carol, U. S. Food and Drug Administration, Cincinnati, Ohio.—No. 1, apomorphine; No. 2, hydrastine, No. 3, theophylline. All the crystals were easily recognized, both from the controls and the descriptions given in the method.

Irwin S. Shupe, U. S. Food and Drug Administration, St. Louis, Missouri.—No. 1, apomorphine; No. 2, hydrastine; No. 3, theophylline. Your description of crystals conformed to those that I obtained. Apomorphine in concentration of 1:50 to 1:150 produced crystals with a drop of 10% HCl. This seemed to be an unusual reaction since most alkaloids are more soluble in acid.

R. D. Stanley, U. S. Food and Drug Administration, Chicago, Ill.—No. 1, apomorphine; No. 2, hydrastine; No. 3, theophylline. An apomorphine hydrochloride solution that had become dark green on standing also produced the diamond-like crystals described in the method.

DISCUSSION

The alkaloids were identified correctly by all the collaborators. The tests for apomorphine and hydrastine are considered adequate for their identification. Shupe observed that crystals are produced by addition of hydrochloric acid to a solution of apomorphine hydrochloride. It is believed that this remarkable reaction may be useful as a confirmatory test.

In the preliminary study, definite crystals in the form of small crosses were obtained by Wagner's reagent with a specimen of pelletierine tannate. The pharmacopoeias[1] describe this product as a mixture of the tannates of the several alkaloids obtained from pomegranate. Therefore, it appears that several specimens from different sources should be examined.

Anabasine yielded no characteristic crystals with the common reagents. According to the literature[2] anabasine is a new alkaloid separated from a Russian plant. It is isomeric with nicotine and is used as an insecticide.

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[1] U. S Pharmacopoeia XI, British Pharmacopoeia 1932.
[2] *C. A.*, **26**, 21, 5699 (1932).

RECOMMENDATIONS[1]

It is recommended–

(1) That the microchemical tests for apomorphine and hydrastine, *This Journal*, **20**, 79 (1937), be made tentative.

(2) That the test for theophylline by mercuric chloride reagent, *Ibid.*, 80, be made tentative.

(3) That Shupe's test for apomorphine by hydrochloric acid reagent be studied next year.

(4) That hydrastinine and cytisine be considered for future study.

(5) That study of anabasine be discontinued.

REPORT ON MICROCHEMICAL METHODS FOR SYNTHETICS

By IRWIN S. SHUPE (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

Tests for the identification of ethylhydrocupreine, barbital, phenobarbital, and amytal were studied this year.

No microchemical methods were found in the literature for the identification of ethylhydrocupreine, but of several papers on the identification of barbital derivatives, those of Denigès,[2] van Itallie and Steenhauer,[3] and Swikker[4] were investigated, and modifications of their tests were used.

In Denigès' method of precipitation of the barbitals from an ammoniacal solution acetic acid was substituted for sulfuric. In addition to the pyridine copper complex used by Swikker, pyridine complexes of cobalt, silver, nickel, zinc, and uranium salts were tried. The copper and silver pyridine salts gave crystalline precipitates with one or more of the barbitals, but the crystal forms varied greatly with the composition and concentration of the solutions. Of the ammonium complexes of nickel, cadmium, cobalt, zinc, copper, and silver, the nickel, cobalt, copper, and silver were found to form crystalline precipitates under certain conditions.

The silver and nickel ammonium complexes produced characteristic crystalline precipitates with barbital and phenobarbital, respectively. No suitable test was found for amytal other than precipitation of the amytal from acid solution.

Ethylhydrocupreine, chemically related to quinine, produced crystals with potassium thiocyanate, but not with other reagents producing crystalline precipitates with quinine. The straight needles with ethylhydrocupreine are readily distinguished from the curved branching crystals with quinine.

The barbital, phenobarbital, and ethylhydrocupreine used for the tests

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 55 (1937).
[2] *Mikrochemie*, **9**, 316 (1931).
[3] *Pharm. Weekblad.*, **67**, 797 (1930); *C. A.*, **24**, 5934 (1930).
[4] *Pharm. Weekblad.*, **68**, 975 (1931); *C. A.*, **26**, 396 (1932).

complied with U.S.P. requirements[1] for purity. The amytal used complied with requirements for purity proposed for it in N.N.R.[2]

Control samples of barbital, phenobarbital, amytal and ethylhydrocupreine, and unknown samples for identification, were sent to collaborators. The unknown samples were (A) Phenobarbital tablets, powdered; (B) amytal tablets, powdered; (C) barbital tablets, powdered; I. quinine, 1:100 in 0.1 *N* HCl; and II. ethylhydrocupreine, 1:100 in 0.1 *N* HCl.

The method used and the characteristics of the synthetics were published in *This Journal*, **20**, 80 (1937).

RESULTS OF COLLABORATORS

The following collaborators, all of the U. S. Food and Drug Administration, reported on the tests as follows:

E. C. Deal, New Orleans, La.—A, phenobarbital; B, amytal; C, barbital.

Using the potassium thiocyanate reagent, I obtained typical crystals in Sample II, indicating ethylhydrocupreine present, but not in Sample I. However, the reaction was a little different than when using an authentic sample. Upon adding the reagent to a 1:100 solution of ethylhydrocupreine in 0.1 *N* HCl, an amorphous precipitate formed instantly. After several minutes needle-like crystals were formed slowly, sometimes with rosettes of small crystals attached to the ends of the needles. In the unknown (No. II) no amorphous precipitate formed, the solution remaining clear upon adding the reagent; only needle crystals formed upon standing, but these needles formed more quickly than they did in the authentic sample.

C. K. Glycart, Chicago, Ill.—A, phenobarbital; B, amytal; C, barbital; I, quinine: Long curved branching crystals unlike the straight needles of ethylhydrocupreine; II, ethylhydrocupreine: Long needles.

The acetic acid reagent produced long needles, also groups of six-sided plates with amytal, dense burrs with phenobarbital, and rectangular crystals with barbital. The ammoniacal silver nitrate reagent and barbital formed double crystals ending in sharp points. The ammoniacal nickel acetate reagent and phenobarbital formed transparent rectangular plates.

The microchemical method is a particularly excellent contribution for the identification of the barbiturates, since the qualitative tests for barbital and phenobarbital heretofore studied, with the exception of the melting points, were found unsuitable for adoption, *This Journal*, **8**, 512 (1925). The unknown powders for identification apparently contained lubricants that were not readily separated by dry extraction with ether as directed. The tests were better when made directly on the powdered tablets.

Morris L. Yakowitz, San Francisco, Calif.—A, phenobarbital; B, amytal; C, barbital; II, ethylhydrocupreine. The crystals described for barbital and phenobarbital are a little difficult to obtain. Careful comparison is necessary.

Paul S. Jorgensen, San Francisco, Calif.—A, phenobarbital; B, amytal; C, barbital; II, ethylhydrocupreine. Amytal and ethylhydrocupreine respond readily to the test. Barbital and phenobarbital respond less readily.

Albert I. Cohen, Chicago, Ill.—A, phenobarbital; B, amytal; C, barbital; I, quinine; II, ethylhydrocupreine. By using authentic known samples as controls, the unknowns are readily identified.

Geo. L. Keenan, Washington, D.C.—The tests for amytal and phenobarbital

[1] U.S.P. XI, pp. 39, 81, 283.
[2] New and Non-Official Remedies, 1934, p. 91.

worked very well and I felt confident that they could be useful in testing for these barbiturates microchemically. I was not so certain of myself in the case of the test for barbital, and if I ventured to make any suggestion, would advise further study on this barbiturate another year.

In the case of amytal, following your directions as given, I found that the crystalline precipitate formed consisted of hexagonal plates and rods, this result being obtained on both the known sample you furnished and Sample B. With phenobarbital, I found that at first a white cloud or amorphous precipitate formed when the reagent was first applied. On stirring, rosette aggregates of needles appeared in the preparation, these being brownish when viewed in mass, and, depending upon the rate of growth, may develop into aggregates of elongated prisms, frequently arranged on edge in the rosette formation and therefore giving the impression of needles. This result was obtained on the known sample and also on Sample A. With partial reliance on your test for barbital as described, I should designate Sample C as consisting of this substance.

DISCUSSION

All the collaborators correctly identified the unknown barbiturates but some reported difficulties. Glycart's comment with respect to lubricants in the unknown was verified. In the Associate Referee's letter to the collaborators it was suggested that they make a dry extraction with ether and test the residues. These residues contained small amounts of stearic acid, which gave greater interference in the tests with the nickel and silver reagents than in those with the acetic acid. When the stearic acid in the residue was removed by barium hydroxide treatment the purified barbiturates responded to the tests more readily but in the same manner as did the controls.

The test for ethylhydrocupreine is considered satisfactory, but with this test as with the other microchemical tests a comparison with controls will often prevent mistakes that might be made if too much reliance is placed on the description of crystals.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical methods presented for the identification of barbital, phenobarbital, amytal, and ethylhydrocupreine be adopted as tentative.

(2) That the following important synthetics be studied: Salicylic acid, benzoic acid, acetylsalicylic acid, saccharin, ethylene diamine, urea, urethane, and other barbituric acid derivatives.

REPORT ON HYPOPHOSPHITES

By Henry R. Bond* (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

This year's work was a continuation of last year's attempt to devise a method for the quantitative determination of hypophosphites in the

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 55 (1937).
* Presented by C. K. Glycart.

presence of phosphates. While circumstances prevented extensive experimentation, a small amount of collaborative work was done in connection with a volumetric method for this determination developed by C. F. Bruening.[1] This method is almost identical with the assay method for ammonium hypophosphite in National Formulary VI, and purports to be applicable in all cases except that of the iron salt, which requires a modification of the method before titration.

Following are the reagents and procedure employed in the method, which involves oxidation of the hypophosphite to phosphate by bromine according to the equation:

$$H_3PO_2+2Br_2+2H_2O \rightarrow H_3PO_4+4HBr.$$

REAGENTS

(a) *Bromide-bromate solution.*—0.1 *N*. Dissolve 3 grams of $KBrO_3$ and 50 grams of KBr in water and dilute to 1 liter.

(b) *Sodium thiosulfate.*—0.1 *N*.

(c) *Potassium Iodide.*—20 grams/100 cc.

(d) *Sulfuric acid.*—10 grams/100 cc.

(e) *Starch solution.*—0.5 gram/100 cc.

PROCEDURE

Accurately weigh about 0.7 gram of the salt and dissolve in enough water to make a final volume of 500 cc. Place an aliquot of 50 cc. in a glass-stoppered, 250 cc. volumetric flask, add 50 cc. of the bromide-bromate solution and 20 cc. of the H_2SO_4, stopper, shake well, and allow to stand for 2 hours. Add 10 cc. of the KI solution, shake the flask, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate until the solution becomes straw color, then add 2 cc. of the starch solution and titrate until the solution becomes colorless. Carry out a blank determination in the same way. The difference between the two titrations with the 0.1 *N* sodium thiosulfate multiplied by the appropriate factor gives the amount of hypophosphite salt present.

For the limited amount of collaborative work performed, two types of samples were selected, the first, ammonium hypophosphite NFV crystals, the product of a reputable manufacturer; the second a sirup of ammonium hypophosphite, a commercial product corresponding in potency to the N.F. product. The ammonium hypophosphite was selected for the purpose of determining the applicability of the method to a simple salt. The sirup was chosen with the idea in mind that the glycerol, sirup, and alcohol, ingredients in the formula, might possibly affect the oxidation to the disadvantage of the method; also, with the thought that, because hypophosphites are usually administered in sirup form, only a method workable on a sirup would be practicable.

The method was applied to both samples by the Associate Referee, whose results indicated that while the two hour oxidation period appeared to be a sufficient length of time for the simple salt, a much longer period

[1] *J. Am. Pharm. Assoc.*, **25**, 19 (1936).

of reaction seemed necessary when the sirup was involved. Accordingly, the collaborators were instructed to employ the original method in one case and to lengthen the reaction period in another when assaying the sirup.

Each collaborator prepared the working sample as follows:

(1) Dried ammonium hypophosphite crystals over H_2SO_4 for 24 hours before weighing sample. Followed outlined procedure from this point.

(2) Pipetted sirup into 50 cc. volumetric flask to mark Rinsed contents of flask into liter volumetric flask, filled to mark with distilled water, and thoroughly shook to make a uniform solution. Used a 25 cc. aliquot of solution for working sample.

Results of the collaborative work are indicated in terms of percentage recovered. In the case of the sirup, percentage of recovery is based on results obtained by the Associate Referee, using the A.O.A.C. tentative method for hypophosphites in the absence of phosphates: 3.464 (3.460) grams of ammonium hypophosphite per 100 cc. All collaborators are members of the Chicago Station, U. S. Food and Drug Administration.

	AMMONIUM HYPOPHOSPHITE CRYSTALS	SIRUP OF AMMONIUM HYPOPHOSPHITE REACTION PERIOD		
		2 HRS.	6 HRS.	20 HRS.
	per cent	*per cent*	*per cent*	*per cent*
E. H. Berry	98.3	90.6	92.2	—
M. Harris	98.67	91.7	96.2	—
	98.65	91.6	96.0	
R. L. Vandaveer	98.5	—	93.2	101.3
	98.0		92 9	100 4
H. R. Bond	98.86	91.6	96.2	—

COMMENTS

Harris.—In the analysis of sirup of $NH_4H_2PO_2$ greater accuracy in amount of sample taken may be possible by use of a weighing bottle and determination of specific gravity. Suggest titration of sample after it has stood 9–12 hours to obtain more complete recovery.

Vandaveer.— Possibly a greater excess of bromine would increase the rate of oxidation.

DISCUSSION

The opinion of the Associate Referee in the light of collaborative results obtained is—

(1) That the NF VI assay for ammonium hypophosphite is probably preferable to the method studied because of the longer reaction period allowed (3 hours instead of 2 hours).[1]

(2) That the accuracy of the method studied depends upon the composition of the product involved when an attempt is made to utilize the

[1] Kolthoff & Furman, Volumetric Analysis, II, 471.

method upon materials other than the simple salt or a mixture of hypophosphites only. The presence of other ingredients appears to have a pronounced effect upon the rate of oxidation. Whether an increase in the length of time of reaction or of the amount of excess bromine, or of both, is necessary, must be determined before the practicability of the method may be assured.

It is the belief of the Associate Referee that until the method studied is sufficiently modified, the present A.O.A.C. tentative method for the determination of hypophosphites in the absence of phosphates may be generally applied with more accurate results in the assay of sirups of hypophosphites.

It is recommended[1] that the method for the determination of hypophosphites by bromine oxidation developed by Bruening be studied further with a view to its modification for adaptation to sirups of hypophosphites.

REPORT ON SANTONIN, PHENOLPHTHALEIN, AND CALOMEL IN TABLETS

By HARRY J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Methods for the determination of all three of the above substances, when occurring separately, have been adopted by this Association. The present problem, therefore, resolves itself into either (1) finding means of separating these compounds preliminary to their determination; or (2) devising methods for the determination of each that are not affected by the presence of the others (or by the presence of other substances occurring in market preparations).

After some experimentation the Associate Referee worked out the following method, which gave satisfactory results in his hands:

PROPOSED METHOD FOR THE DETERMINATION OF SANTONIN, PHENOLPHTHALEIN, AND CALOMEL IN TABLETS

PREPARATION OF SAMPLE

Weigh 4 grams of the powdered material into a Caldwell crucible and wash with about 200 cc. of hot alcohol, collecting the filtrate in a 250 cc. volumetric flask, using a bell-jar and suction. Make the filtrate to volume at 20°C. Designate this solution as "A" and the residue in the crucible as "B."

DETERMINATION

(a) *Santonin.*—Treat a 25 cc. aliquot of "A" by the tentative method for santonin in mixtures, *Methods of Analysis, A.O.A.C.*, 1935, 588, 130, beginning with "add 50 cc. of dinitrophenylhydrazine soln."

(b) *Phenolphthalein.*—Evaporate a 50 cc. aliquot of "A" to dryness, and proceed as directed in *Methods of Analysis, A.O.A.C.*, 1935, 570, 80.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 55 (1937).

TABLE 1.—*Santonin-Phenolphthalein-Calomel Mixture*
(Results expressed as per cent)

COLLABORATOR	SANTONIN					PHENOLPHTHALEIN					CALOMEL			
	1	2	3	4	AVERAGE	1	2	3	4	AVERAGE	1	2	2	AVERAGE
S. W. Bower Univ. of Buffalo Buffalo, N. Y.	16.65	16.60	16.79		16 68	18.20	18 05	17.81		18.02	6.47	6.50	6.56	6.51
H. M. Burlage Univ. of North Carolina Chapel Hill, N. C.	16.88	16.78	16.95	16.95	16.89	20.69	20.69	20.87		20.75	6.56	6.42		6.49
W. F. Reindollar State Dept. of Health Baltimore, Md.	17.10	17.19	16.40	16 45	16.79	17.35	17.95	17.91	17.68	17.72	6.64	6.47		6.56
H. E. Sayles The S. E. Massengill Co. Bristol, Tenn.	17.14				17.14	17.61				17.61	6.20			6.20
I. S. Shupe U. S. Food and Drug. Adm. St. Louis, Mo.	16.7	16.5			16.6	16.6	16.8			16.7	6.45	6.45		6.45
H. J. Fisher	16.56	16.73	16.57	16.53	16.60	16.49	16.28	16.53		16.43	6.21	6.16		6.19
Average					16.78					17.87				6.40
Theoretical					16.70					16.70				6.47

(c) *Calomel.*—Wash "B" with three 15 cc. portions of cold water, using suction, and proceed as directed in *Methods of Analysis, A.O.A.C.*, 1935, 595, 152, beginning with "Transfer the removable plate."

A sample was prepared and sent to six collaborators with directions that it be analyzed by this method. The composition of the sample, which was designed to simulate that of commercial santonin-phenolphthalein-calomel tablets, was as follows:

	per cent
Santonin	16.70
Phenolphthalein	16.70
Calomel	6.47
Corn starch	12.53
Acacia	4.18
Lactose	34.39
Orange I	0.13
Saccharin	0.43
Talcum	8.35
Oil of lemon	0.12
	100.00

Five collaborators reported. Their results are summarized in Table 1. The following conclusions may be drawn from the table:

(1) The method gives excellent results for santonin and calomel. Phenolphthalein does not interfere in the determination of santonin as the dinitrophenylhydrazone.

(2) Most analysts obtained high results for phenolphthalein by the method. The precipitation of phenolphthalein as the tetraiodo compound does not effect a sharp separation from santonin.

It is recommended[1] that this study be continued, and that particular attention be given to the determination of phenolphthalein in the presence of santonin.

REPORT ON BENZYL COMPOUNDS

By S. Reznek (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

The work of the previous Referee on Benzyl Compounds consisted of the development of methods for determining benzyl alcohol in water and salt solutions and in ethyl alcohol solutions, *This Journal*, 16, 285 (1933). The former method is also adaptable to the estimation of a benzyl compound such as the succinate, which may be saponified by aqueous alkali and the benzyl alcohol determined by distillation from the mixture. The next most frequently used benzyl compound is probably benzyl benzoate, administered in solution in a fixed oil. Methods based on saponification followed by distillation of the alkaline solution were unsuccessful. Saponification in aqueous alkali is incomplete. Attempts were made to determine

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 55 (1937).

the benzoic acid by distilling from acid solution after saponifying with alcoholic alkali or glycerol-soda, and acid hydrolysis with various strengths of sulfuric acid, but incomplete recovery was obtained even on continued steam distillation.

The method finally evolved depends on the relative solubility of benzoic acid in water compared to the insolubility of oleic acid in water. The water solution of benzoic acid may be freed of oleic acid (arising from the saponified fatty oil) by filtration and the benzoic acid extracted and titrated. The oleic acid is washed with hot water to free it of any dissolved benzoic acid.

If the fixed oil used as a vehicle for the benzyl benzoate is known to be olive oil, as is usually the case, the above determination may be checked by use of the Abbé refractometer reading as described below.

In the experimental work benzyl benzoate of good quality was redistilled, and the fraction boiling at 325–327°C. was used. The refractive index was 1.5665 at 25°C., specific gravity 1.120 at 20°/20°, and the benzyl benzoate content (from the saponification value), 99.6 per cent. Mixtures were made by pipetting the desired volume of benzyl benzoate into volumetric flasks and making up to volume with a good grade of olive oil. The refractive indices of various mixtures are given in the table.

Abbé refractometer readings of solutions of benzyl benzoate in olive oil

BENZYL BENZOATE/100 CC. SOLUTION	REFRACTOMETER READING AT 25°C.
cc.	
0 (pure olive oil)	1.4667
2	1.468
4	1.470
10	1.478
25	1.492
50	1.517
Pure benzyl benzoate	1.567

The amount of benzyl benzoate in olive oil solution, when the solution consists of these two components only, may be calculated within 1 or 2 per cent from the formula: Per cent benzyl benzoate $=100-[1000(1.567-r)]$, where "r" is the Abbé refractometer reading at 25°C. The formula is based on the value 1.467 for olive oil. The refractive index for olive oil is given as 1.466–1.467.[1] This range is sufficiently small so that use of the upper figure to simplify the formula does not materially affect the accuracy of the calculation. Of course, the formula applies only when it is known that olive oil is the solvent used.

The method follows:

SAPONIFICATION METHOD

Pipet 5 cc. of sample into a 400 cc. beaker, add 50 cc. of 5% alcoholic NaOH or KOH, cover with a watch-glass, and place on the steam bath for 30 minutes. Re-

[1] Handbook of Chemistry and Physics, p. 768 (20th Ed.).

move the watch-glass and evaporate nearly to dryness to remove most of the alcohol. Dissolve the residue in 150–200 cc. of warm water and cool to room temperature. Make distinctly acid to litmus with 10 per cent H_2SO_4 and let stand to allow the fatty acids to separate. Warm gently (40°–50°), but avoid heating to a point sufficient to vaporize any of the benzoic acid. Pour into a 250 cc. separator and allow the oily layer to separate. Draw off the aqueous layer into a 500 cc. volumetric flask and avoid allowing any of the fatty acids to run into the flask. Shake the fatty acids with 50–100 cc. of hot water, allow to separate, and add the aqueous layer to the contents of the volumetric flask. Repeat the shake-out with hot water twice more, allow the combined aqueous layers to cool, and make up to 500 cc. Filter through a double filter to remove droplets of fatty acid. Pipet a convenient aliquot—100 cc. when 5 cc. of 25–50% benzyl benzoate solution is taken for analysis—into a separator, saturate with salt, and shake with one 50 cc. and four 25 cc. portions of $CHCl_3$. Draw off the $CHCl_3$ layer into a second separator, wash the combined first three extracts with 5 cc. of water, allow to separate, and filter through cotton wet with $CHCl_3$ into a 400 cc. beaker. Combine the last two extracts, wash with the same 5 cc. of water, filter, and add to the first $CHCl_3$ extracts. Evaporate all except 25 cc. of the $CHCl_3$ at a temperature not above 75° C., then evaporate to dryness at not above 40° C.

Dissolve the benzoic acid in neutral alcohol and titrate with 0.1 *N* alkali, using phenolphthalein indicator. 1 cc. of 0.1 *N* alkali = 0.0212 gram of benzyl benzoate, or 0.0191 cc. of benzyl benzoate at 20°C. (based on specific gravity of 1.114).

A commercial sample consisting of ampuls labeled as containing 0.5 cc. of benzyl benzoate made up to 1 cc. with olive oil gave 48 per cent benzyl benzoate by refractometer and 47.6 per cent by the saponification method. Samples containing 25 per cent and 50 per cent benzyl benzoate (v/v), respectively, were analyzed by W. E. Kirby of the New York Station, who obtained the following results:

	Benzyl Benzoate Found	
	By Saponification	*By Refractometer*
	per cent	*per cent*
25% Solution	23.0	25.0
50% Solution	44.4	50.0

It is recommended[1]—

(1) That the above refractive index and saponification methods be adopted as tentative.

(2) That because of the lessened importance of benzyl compounds, as shown by their omission from recent editions of the N.N.R., further work on these compounds be discontinued.

REPORT ON RHUBARB AND RHAPONTICUM

By A. VIEHOEVER (Philadelphia College of Pharmacy and Science, Philadelphia, Pa.) *Associate Referee**

In the 1937 Supplement of the U. S. Pharmacopoeia, *Rheum rhaponticum* is specifically excluded as an official source of rhubarb.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 55 (1937).
* From the Gross Laboratory for Biological and Biochemical Research.

The chemical tests of identity, representing the so-called Borntraeger color reaction, indicating the presence of emodin and chrysophanic acid, are given by both rhubarb and rhaponticum. Rhaponticin has been found to occur in genuine rhubarb as well as in rhaponticum. No reliable chemical identity tests, nor indeed chemical assay methods are at present available for the differentiation of rhubarb from rhaponticum and for the evaluation of rhubarb.

The complex chemical composition thus far known has been enumerated in a previous report by this Referee, *This Journal*, **16**, 530 (1933). It has also been pointed out in this and other reports.[1] that by using daphnia as a biological test animal a difference has been found in the cathartic effectiveness of both species, rhaponticum having been found weaker than rhubarb. Daphnia for these experiments have been successfully bred in aerated tapwater, containing either 125 mg. of soybean flour (Cellu) and 25 mg. of urea to each gallon, or at most 0.1 per cent of dried shredded sheep manure,[2] the latter medium usually yielding darker pigmented and thus more conspicuous contents in the food canal.

In order to express the cathartic effectiveness in a definite quantitative way, elaterin has been used as a standard substance. The commercial substance elaterin is a combination of two crystalline compounds: elaterin α, crystallizing in prisms and melting above 200°C. (230°C. or less), levorotatory, physiologically inactive, not readily soluble in absolute alcohol; and elaterin β, crystallizing in plates melting below 200°C. (190-195°C.(?)), dextrorotatory, readily soluble in absolute alcohol, and physiologically a very active cathartic.

While the complete separation has not been effected, the mixture, upon suspension in culture water, as well as the filtrate of this suspension (a fairly coarse filter paper used), has caused speedy evacuation of the entire food canal of daphnia. The speed of complete evacuation, effected generally in 10–20 minutes, markedly exceeded that observed previously for curacao aloe in a concentration of 0.05 per cent.[3]

A tentative assay is suggested for the biological evaluation of rhubarb and rhaponticum, in particular, and for laxative drugs in general. Consideration was given—

I. To the standardization of conditions, as
   1. Number of animals.
   2. Amount of culture water.
   3. Composition including the pH concentration of the culture water.
   4. Temperature of the culture water.
   5. Pretreatment of the animal (pure line, uniform sex and age).
   6. Manner of administration of the preparation to be tested.

II. Determination of the normality spread of normal deviation, as
   1. The best time interval of observation.

[1] *J. Am. Pharm. Assoc*, **25**, No 12 (1936)
[2] *Am. J. Pharm*, **107**, 103–130 (1935), **109**, 285–316 (1937).
[3] Viehoever, A., *Am. J. Pharm.*, **107**, 47–72 (1935).

2. Intensity of effect at a certain prefixed time.

III. Unit of effect (fixed in terms of time and effect).

IV. Variation of reaction of animals.

The Associate Referee will submit the proposed assay method to collaborators. Continuation of the work is recommended.

The method follows:

ASSAY METHOD FOR THE EVALUATION OF LAXATIVES

TEST OBJECT

Daphnia (*Daphnia magna*).—Age 10 days. Reared from pure strains, at temperatures between 68°–72°C. in culture water of known *p*H concentration (*p*H 7.8–8.1); female (without eggs or young in the brood sac) from the same colonies, kept under identical conditions and with 100% filled food canal. Use 30 daphnia for each test.

PROCEDURE

Prepare a suspension of reference elaterin (Merck) in concentration of 0.1 gram in 100 cc. of culture water of *p*H 7.8–8.1 and filter through fairly coarse filter paper.* Preferably use crystalline elaterin β, melting point 197°C., 0.02 gram in 100 cc. of the culture water, and filter. If the solution obtained is to be preserved, check it for potency against freshly prepared solutions at least every month and discard it if not of standard potency.

Add the standard in 0.5 cc. quantities to the test animals, placed singly in special observation tubes (flat, 3″ long, 0.5″ wide) freed from decantable culture water.

Observe the speed and extent of evacuation at low magnification (6–10), and check, if desirable, with higher magnification (100). Record the time of approximately 50% and 100% evacuations.

To test the preparation, dissolve or suspend it in amounts of 0.1 cc. in 10, 50, and 100 cc. of the same culture water as specified above and filter. Add this test solution in 0.5 cc. quantities to the test animals. Proceed as directed for the standard solution.

Determine the concentration of the test and standard solutions producing the same degree of response.

REPORT ON HEXYLRESORCINOL

By Morris L. Yakowitz (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

The former referee on this subject worked out a bromination procedure for the assay of pure hexylresorcinol. His report of collaborative results, *This Journal*, **16**, 384 (1933), indicated that the method needed some revision in order to give concordant results in the hands of other workers. Therefore a study was made of this method.

Hexylresorcinol, while soluble in alkalies, is insoluble in neutral and acid solutions. The method referred to specifies the dissolving of 50 mg. of sample in 2 cc. of alkali and the addition of 30 cc. of bromine solution, followed by the addition of 10 cc. of hydrochloric acid. It was found that when water was substituted for the 30 cc. of bromine solution, the final acidification threw the hexylresorcinol out of solution. This indicated a

* Delta, No. 307 or Amer. Standard, No. 5160.

possible reason for the low results obtained in the original bromination experiments. It was thought that if some organic solvent could be used so that the hexylresorcinol would remain in solution when the final dilution was made, higher results should be obtained. Preliminary experiments indicated that commercial ethyl alcohol could not be used for this purpose since the alcohol itself consumed a considerable amount of bromine. Methyl alcohol, however, was found to consume but a small amount of bromine; this consumption seemed to depend upon the particular brand of methyl alcohol used and to indicate that it was due to a contaminant of the methyl alcohol and not to the alcohol itself. Commercial methyl alcohol was taken, sufficient bromine vapor was added to color it a bright yellow, and the mixture was boiled on the steam bath for 5 minutes. The solution was cooled and rendered colorless by the careful addition of a concentrated sodium acid sulfite solution. Methyl alcohol so treated did not consume any bromine in the method as finally developed.

Experiments showed that when 90 mg. of hexylresorcinol was dissolved in 10 cc. of methyl alcohol and diluted with 30 cc. of water and 5 cc. of hydrochloric acid, all the hexylresorcinol remained in solution.

The method as finally developed for the bromination of hexylresorcinol was published in *This Journal*, **20**, 81 (1937).

The results obtained by the Referee and his collaborators are as follows:

Name	*Recovery per cent*			
M. L. Yakowitz	98.9	99.0		
P. S. Jorgensen F. & D. Adm., San Francisco	98.3	98.3		
J. A. Kime F. & D. Adm., San Francisco	98.9	98 1		
H. G. Underwood F. & D. Adm., Cincinnati	98.72	98.46	98 23	
I. S. Shupe F. & D. Adm., St. Louis	97.7	98.3	98.8	98.3

Excluding the Associate Referee's results the collaborators found an average recovery of 98.37 per cent, the lowest recovery being 97.7 and the highest recovery 98.9 per cent.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the assay of pure hexylresorcinol be adopted as a tentative method.

(2) That further work be done to evolve methods for determining hexylresorcinol in its commercial preparations and also to find methods for the identification of hexylresorcinol in preparations of secret composition.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937).

REPORT ON CHEMICAL ASSAY FOR ERGOT ALKALOIDS

By C. K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Pursuant to the recommendation made last year, this subject was continued. A method for the determination of the recently discovered water-soluble alkaloid of ergot was devised this year by Hampshire and Page.[1] After an extensive investigation of ergot, which included a comparative study of various solvents with ergotoxine and ergometrine, they reported an assay process in which the water-soluble alkaloids are separated from the water-insoluble alkaloids.

Briefly stated, the method used consists of two parts: (1) A sample of defatted ergot rendered faintly alkaline by ammonia is exhausted with alcohol-free ether in a continuous extraction apparatus. The ammoniacal ethereal extract is made to a known volume, one aliquot is assayed for total alkaloids by shaking with successive portions of a solution of tartaric acid and comparing the blue color obtained by reagent *p*-dimethylaminobenzaldehyde with a standard solution of ergotoxine by means of a colorimeter. (2) The other aliquot of the ether solution is shaken with successive portions of water until the ergometrine is completely removed and then similarly assayed for residual water-insoluble alkaloids. The results of these two assays are calculated as ergotoxine, and the difference multiplied by a conversion factor is ergometrine.

This factor, it was stated, was derived by Allport and Crews,[2] who found that the relative color intensity produced by ergometrine and dimethylaminobenzaldehyde reagent is 1.86 times that of ergotoxine, also that the colors are spectroscopically identical when made under similar conditions.

The work this year included a study of the Hampshire-Page method adapted to the assay of fluidextracts; the application of the wedge photometer, recently devised by Clifford;[3] a comparison of the results obtained by the photometer and the micro-colorimeter; and also the separation and estimation of water-soluble alkaloids in a commercial sample of tablets by means of choloroform and carbon tetrachloride.

The material for the preliminary work consisted of three commercial samples of fluidextract of ergot of different manufacture. Samples A and B were over two years old. Sample C was labeled U.S.P. XI.

CHEMICAL ASSAY FOR ALKALOIDS OF ERGOT

REAGENTS

(a) *Alcohol-free ether.*—Shake U.S.P. ether (peroxide-free) with several successive portions of distilled water in a separator and discard the aqueous layer.

(b) *Tartaric acid solution.*—1%.

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[1] *Quart. J. Pharm. Pharmacol.* 9, 60 (1936).
[2] *Ibid.*, 8, 447 (1935)
[3] *This Journal*, 19, 130 (1936).

(c) *Modified Smith reagent.*—Dissolve 0.125 gram of *p*-dimethylaminobenzaldehyde in a cooled mixture of 65 cc. of concentrated H_2SO_4 and 35 cc. of H_2O and add 0.015 gram of ferric chloride, U.S.P. (reagent must not be used after 7 days).

(d) *Standard solution of ergotoxine.*—0.012 % solution in a 1% solution of tartaric acid in water, 1 cc. containing the equivalent of 0.0001 gram of anhydrous ergotoxine.

PROCEDURE

Pipet 10 cc. of the fluidextract at 20°C. into a small beaker and dealcoholize on the water bath at 60°C. Transfer with water to a continuous extraction apparatus. (A Palkin-Murray-Watkins automatic extractor[1] is suitable.) Add 2 cc. of strong ammonia, and continue the extraction with the washed ether for 4–5 hours. Disconnect the flask and filter the ethereal extract through a small filter paper and wash the flask with small portions of the ether from the extractor into a 100 cc. volumetric flask. Adjust to mark at 20°C.

Total Alkaloids.—Transfer a 50 cc. aliquot to a separator and shake with four successive 10 cc. portions of the aqueous 1% tartaric acid solution or until the alkaloids are removed completely. Test for complete extraction by shaking with a further portion of the tartaric acid solution until 2 cc. shows no blue color with Smith reagent.

Warm the combined acid solution on a water bath in a current of air to remove the ether; transfer the solution to a 50 cc. volumetric flask, cool, and make to volume with 1% tartaric acid solution.

Water-insoluble Alkaloids.—Shake the remaining 50 cc. aliquot of the ethereal extract with successive 20 cc. portions of water made faintly alkaline with ammonia until 1 cc. of the separated aqueous liquid yields no blue color when mixed with 2 cc. of modified Smith reagent. Shake the ethereal solution with four 10 cc. portions of the tartaric acid solution (test for complete extraction by shaking with a further portion of the tartaric acid solution until 2 cc. shows no blue color with the Smith reagent), warm the combined acid solution to expel the ether, and transfer to a 50 cc. flask; cool and make to mark with the tartaric acid solution.

Colorimetric Determinations

Pipet 1 cc. of the standard ergotoxine solution at 20°C. into a glass colorimetric cup or small test tube and add 2 cc. of the modified Smith reagent. Mix. Pipet 1 cc. each of the ergot solutions into cups or test tubes and add the reagent in a similar manner. Allow to stand 15 minutes and read in a colorimeter.

Calculate the total alkaloids and water-insoluble alkaloids as ergotoxine. Multiply the difference by 0.538 for ergometrine.

Photometric Determination

STANDARDIZATION

Prepare a series of solutions containing 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 cc. of the standard solution of ergotoxine and add 1% tartaric acid sufficient to make 1 cc. exactly. Add 2 cc. of the modified Smith reagent, mix, and let stand 15 minutes. Prepare a blank solution containing 1 cc. of the 1% tartaric acid and 2 cc. of the modified Smith reagent to correct for incidence of the glass surface of the cell and plot as zero point. Plot a graph from the readings obtained, using a monochromatic filter (No. 66 is suitable).

DETERMINATION

Pipet 1 cc. of ergot solutions into a test tube. Mix with 2 cc. of the modified Smith reagent. Let stand 15 minutes. Fill the cell and ascertain the reading on the photometer scale. Determine the percentage of ergotoxine by reference to the graph.

[1] *J Ind Eng. Chem*, **17**, 612 (1925).

The results follow:

SAMPLE FLUIDEXTRACT OF ERGOT		TOTAL ALKALOIDS AS ERGOTOXINE	WATER-INSOLUBLE ALKALOIDS AS ERGOTOXINE	WATER-SOLUBLE ALKALOIDS BY DIFFERENCE AS ERGOTOXINE A	WATER-SOLUBLE ALKALOIDS AS ERGOMETRINE (A×0.538)
		per cent	*per cent*	*per cent*	*per cent*
A	Colorimeter	0.065	0.060	0.005	0.003
	Photometer	0.064	0.054	0.010	0.005
B	Colorimeter	0.069	0.057	0.012	0.006
	Photometer	0.064	0.050	0.015	0.008
C	Colorimeter	0.071	0.048	0.023	0.012
	Photometer	0.072	0.051	0.021	0.011
C*	Colorimeter	0.081	0.053	0.028	0.015
	Photometer	0.074	0.048	0.026	0.014

* Assay made by K. L. Milstead, U. S. Food and Drug Administration, Chicago, Ill.

SEPARATION AND ESTIMATION OF ERGOTOXINE AND ERGOMETRINE IN TABLETS

According to Hampshire and Page, ergometrine is not extracted from ammoniacal solution by carbon tetrachloride, but ergotoxine is readily extracted by this solvent.

A commercial sample of tablets was assayed by the following method:

Weigh a sufficient number of tablets to contain 0.005 gram of the alkaloidal salt. Treat with 20 cc. of the 1% solution of tartaric acid. Filter, transfer to a separator, and make faintly alkaline with ammonia. Extract with 5 successive portions of carbon tetrachloride. Shake the combined solvent with 1% solution of tartaric acid and make to volume.

Determine ergotoxine by comparison with the standard solution. Transfer the aqueous solution remaining in the separator to a volumetric flask, and make to volume with 1% tartaric acid solution. Determine the alkaloid by comparison with a standard solution of ergotoxine. Multiply by 0.538 for conversion to ergometrine.

SUMMARY

The separation of the new alkaloid by means of water is a simple and direct procedure, providing both qualitative and quantitative information, and merits further study. The results of the preliminary assays indicate that the neutral wedge photometer is adaptable to the determination of ergot alkaloids. This instrument is designed to measure small quantities. It eliminates frequent preparation of standard solutions, and only a single calibration with the standard ergotoxine solution is necessary.

It is recommended[1] that the methods for ergot be further studied.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937).

REPORT ON NITROGLYCERIN IN MIXTURES

By OMER C. KENWORTHY* (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

Nitroglycerin has long been used in the form of hypodermic or compressed tablets. In the past few years, however, its use in combination with other medicinal ingredients such as caffeine, reduced iron, and extractives from hyoscyamus, belladonna, nux vomica, etc., has become popular. A study of the determination of nitroglycerin in such mixtures has been found necessary in view of the fact that the present A.O.A.C. methods, *Methods of Analysis, A.O.A.C.*, 1935, (ether extraction and alcohol aliquot) have been found inapplicable when the tablets contain any other nitrogenous matter. The colorimetric methods are likewise unreliable.

For the purpose of this study the method developed by Anderson (acid distillation method), *This Journal*, **15**, 140 (1932), offered the most promise since it depends upon distilling the nitroglycerin from the other ingredients. It was used with some modifications.

The present report covers work on the determination of nitroglycerin in the presence of caffeine, reduced iron, and tinctures of digitalis, nux vomica, belladonna, hyoscyamus, and strophanthus, each singly and in various combinations. In each case the amount used was equal to or greater than the amount that would be present in a sample of the complex tablets. In the case of the tinctures, the alcohol was not evaporated because it does not interfere with the determination.

An alcoholic solution of nitroglycerin was used as it would be more uniform in composition. A sample of spirit of nitroglycerin was purchased on the open market, assayed, and diluted with alcohol so that 5 cc. of the solution would contain approximately one-half grain of nitroglycerin. The method follows:

Weigh or measure the prescribed "added" materials to an 800 cc. Kjeldahl flask. Add 50 cc. of a saturated solution of Na_2SO_4, make acid to litmus with 10% H_2SO_4, and then add 0.5–1 cc. in excess, 5 cc. of the nitroglycerin solution, and 150 cc. of water. Distil just to dryness, using a distilling trap, into an 800 cc. Kjeldahl flask containing 30 cc. of 5% NaOH, keeping the outlet tube below the surface of the alkali. Use a moderate flame throughout so that no nonvolatile material is carried over by spray. Disconnect the flask and wash the condenser and outlet tube with about 100 cc. of water, receiving the washings in the flask containing the alkali. Add 2 grams of Devarda alloy and distil the ammonia into 25 cc. of 0.02 *N* H_2SO_4, using a spray type distilling trap Titrate the excess acid with 0.02 *N* NaOH, using methyl red indicator. Use care in the ammonia distillation as there is considerable foaming during the early part of the distillation.

1 cc. of 0.02 *N* H_2SO_4 = 0.001514 gram of nitroglycerin.

* Presented by F C. Sinton.

RESULTS

	gram	
5 cc. nitroglycerin solution alone	0.0293	
	0.0287	
	0.0292	
	0.0289	
	0.0287	
Av.	0.02896	
5 cc. nitroglycerin solution +	0.0295	
10 cc. tincture of digitalis	0.0295	
Av. Recovery		101.9%
5 cc. nitroglycerin solution +	0.0294	
25 cc. tincture of digitalis	0.0294	
Av. Recovery		101.5%
5 cc. nitroglycerin solution +	0.0291	
10 cc. tincture of hyoscyamus	0.0288	
Av. Recovery		100.0%
5 cc. nitroglycerin solution +	0.0292	
25 cc. tincture of hyoscyamus	0.0293	
Av. Recovery		101.0%
5 cc. nitroglycerin solution +	0.0287	
10 cc. tincture of belladonna leaves	0.0290	
Av. Recovery		99.6%
5 cc. nitroglycerin solution +	0.0288	
25 cc. tincture of belladonna leaves	0.0292	
Av. Recovery		100.1%
5 cc. nitroglycerin solution +	0.0288	
10 cc. tincture of nux vomica	0.0287	
Av. Recovery		99.3%
5 cc. nitroglycerin solution +	0.0286	
25 cc. tincture of nux vomica	0.0288	
Av. Recovery		99.1%
5 cc. nitroglycerin solution +	0.0286	
10 cc. tincture of strophanthus	0.0289	
Av. Recovery		99.3%
5 cc. nitroglycerin solution +	0.0287	
25 cc. tincture of strophanthus	0.0286	
Av. Recovery		98.9%
5 cc. nitroglycerin solution +	0.0287	
1 gram caffeine	0.0289	
Av. Recovery		99.4%
5 cc. nitroglycerin solution +	0.0291	
5 grams caffeine	0.0293	
Av. Recovery		100.8%
5 cc. nitroglycerin solution +	0.0300	
1 gram reduced iron	0.0294	
Av. Recovery		102.6%
5 cc. nitroglycerin solution +	0.0298	
5 grams reduced iron	0.0299	
Av. Recovery		103.1%
5 cc. nitroglycerin solution +		
1 gram caffeine		
1 gram reduced iron	0.0293	

Recovery		101.1%
5 cc. nitroglycerin solution +		
1 gram caffeine		
10 cc. tincture digitalis		
10 cc. tincture hyoscyamus		
10 cc. tincture nux vomica		
10 cc. tincture belladonna leaves		
10 cc. tincture strophanthus	0.0278	
Recovery		96.0%
5 cc. nitroglycerin solution +		
1 gram reduced iron		
10 cc. tincture digitalis		
10 cc. tincture hyoscyamus		
10 cc. tincture nux vomica		
10 cc. tincture belladonna leaves		
10 cc. tincture strophanthus	0.0430	
Recovery		148.5%
5 cc. nitroglycerin solution +		
1 gram caffeine		
1 gram reduced iron		
10 cc. tincture digitalis		
10 cc. tincture hyoscyamus		
10 cc. tincture nux vomica		
10 cc. tincture belladonna leaves		
10 cc. tincture strophanthus	0.0453	
Recovery		156.5%
5 cc. nitroglycerin solution +		
10 cc. tincture digitalis		
10 cc. tincture hyoscyamus		
10 cc. tincture nux vomica		
10 cc. tincture belladonna leaves		
10 cc. tincture strophanthus	0.0286	
Recovery		98.8%

CONCLUSIONS

The acid distillation procedure appears to be promising as a method for determining nitroglycerin in mixtures. When used separately, none of the materials investigated interfered with the recovery of the nitroglycerin.

Reduced iron in the presence of the tinctures gave high results. Whether it was due to only one of the tinctures or to all of them is not known at the present time. It is recommended[1] that the work be continued.

No report on guaiacol was given by the associate referee.

No report on biological testing was given by the associate referee.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937)

REPORT ON IODINE OINTMENT

By W. F. Reindollar (Department of Health, Baltimore, Md.), *Associate Referee*

The efforts of the Associate Referee during the past year were devoted to the determination of iodine, iodide, and organically combined iodine in iodine ointment. Assays were made on an old ointment prepared in April, 1934 and recently remixed, and on fresh ointment prepared just prior to the tests.

Iodine.—Three methods for the estimation of this constituent were studied. The first involved distillation of the halogen and subsequent titration, the two latter titration of the iodine in the presence of the base, in one case with 0.1 *N* sodium thiosulfate and in the other with 0.1 *N* potassium arsenite solution. The results follow:

	Distillation	Titration	
		0.1 *N* $Na_2S_2O_3$	0.1 *N* K_3AsO_3
	per cent	*per cent*	*per cent*
Old ointment	2.96	3.29	3.21
	3.04	3.29	3.26
Fresh ointment		3.90	3.91
		3.94	3.91
		3.97	3.97
		3.97	3.95

The values obtained by the distillation method are quite low, but those by the titration procedures are rather satisfactory. As the alkaline arsenite solution may be prepared from arsenic trioxide, a primary standard, and once made retains its titer, it is believed to be a more satisfactory reagent than the sodium thiosulfate solution. The procedure involving its use is described as follows:

IODINE IN IODINE OINTMENT

Weigh accurately 4–5 grams of the ointment in an iodine flask, stopper, and heat on a water-bath until the sample is just fluid. Add 30 cc. of $CHCl_3$ and shake with a rotary motion until the base is dissolved. Add 30 cc. of water and titrate immediately with 0.1 *N* $KAsO_2$ solution (containing sufficient $KHCO_3$ to neutralize the HI formed), employing starch as the indicator. 1 cc. of 0.1 *N* $KAsO_2$ = 0.012692 gram of I.

Potassium Iodide.—The following procedures were studied in an attempt to estimate the iodide content:

(a) Volatilization of free iodine by boiling, liberation of the combined iodine by adding sulfuric acid and ferric ammonium alum, distillation, and subsequent titration of the halogen.
(b) Distillation without previous volatilization, titration of halogen, and calculation of potassium iodide by difference.
(c) Removal of uncombined iodine and iodide from the base by repeated washings, volatilization of free iodine, and titration of the iodide by the Volhard method.

(d) Procedure similar to (c) except that the iodide was titrated with 0.05 *M* potassium iodate solution.

Of these methods both (c) and (d) yielded results that were in good agreement, but about 5 per cent high. Method (d) is described as follows:

POTASSIUM IODIDE IN IODINE OINTMENT

Weigh accurately 4–5 grams of the ointment in a 100 cc. beaker, add 50 cc. of water, and heat on a water bath until fluid, stirring occasionally. Remove the beaker, immerse in cold water until the base has solidified, and pour the aqueous layer into an iodine flask. Repeat the extraction with decreasing quantities of water (25 cc. and 4 or 5 10 cc. portions) until all the iodide and iodine are removed. Boil the combined washings until all the free iodine has been volatilized and the volume is about 25 cc. Add 60 cc. of concentrated HCl and 5 cc. of $CHCl_3$ and titrate with 0.05 *M* potassium iodate solution. 1 cc. = 0.01660 gram of potassium iodide.

The following results were obtained by this method:

	Potassium Iodide Contained *per cent*	Found *per cent*	Average Recovery *per cent*
Old Ointment	4.0	4.25	106.2
		4.26	
Fresh Ointment A	4.0	4.29	105.5
		4.23	
		4.15	
Fresh Ointment B	4.07	4.27	104.2
		4.23	
		4.25	
		4.20	

Believing that the high results were caused by partial conversion of free iodine to iodide by the base, the Associate Referee assayed by the same method an ointment containing 4 per cent iodine and no potassium iodide. It was found to contain 0.28 per cent of iodide-iodine.

Fixation of the free iodine may occur in two ways: (1) By reacting with free alkali introduced by the lanolin, and (2) by being reduced to hydriodic acid by one of the constituents of the base. That the latter is the case was shown by titrating the iodine-free washings with 0.1 *N* sodium hydroxide. They were found to contain about 0.3 per cent hydriodic acid. Further investigation of this subject indicates that the major part of this reduced iodine does not exist in the ointment, but is produced by the manipulations of the assay. Hence, it appears that the accurate estimation of the potassium iodide must depend upon a quantitative determination of the cation.

Organically Combined Iodine.—The amount of iodine absorbed by the base is small, but it increases with the age of the ointment. Old and fresh ointments, and a base composed of the mixed fats and serving as a control, were run by the following method.

ORGANICALLY COMBINED IODINE IN IODINE OINTMENT

Weigh accurately 4–5 grams of the ointment in a 100 cc. beaker, add 50 cc. of water, and heat on a water bath until fluid, stirring occasionally. Remove the beaker, immerse in cold water until the base has solidified, and pour the aqueous layer into an iodine flask. Repeat the extraction with decreasing quantities of water (25 cc. and 4 or 5 10 cc. portions) until all the iodide and iodine are removed. Determine iodine in the separated base by the method given in United States Pharmacopoeia XI for the determination of total iodine in iodine ointment.

The following results were obtained by this method when freshly prepared iodine ointment was used: 0.09, 0.13, 0.12, and 0.12 per cent.

RECOMMENDATIONS[1]

It is recommended—

(1) That the determination of iodine in iodine ointment be studied collaboratively with a view to its adoption as a standard method.

(2) That the determination of potassium iodide in iodine ointment be further studied.

(3) That the method for organically combined iodine be studied collaboratively.

REPORT ON ACETPHENETIDIN IN THE PRESENCE OF CAFFEINE AND ASPIRIN

By SOLOMON M. BERMAN (U. S. Food and Drug Administration, Philadelphia, Pa.), *Associate Referee*

No collaborative work was done on this topic during the past year. On recommendation of the Referee, the scope of the topic was extended with the object of developing a satisfactory caffeine determination.

Experiments were made on a known mixture to test the effect of sample size on the caffeine recovery. The mixture was made up to contain 47.4 per cent of aspirin, 47.5 per cent of acetphenetidin, and 2.50 per cent of anhydrous caffeine. The caffeine was introduced as caffeine citrate.

The aspirin was separated from the mixture by the method published in the previous report, *This Journal*, **19**, 520 (1936), with the slight change of using 10 cc. of the bicarbonate solution in the initial extraction for the pair of largest samples. The acetphenetidin was separated by the hydrolysis procedure under the heading "Gravimetric Method," except that the sulfuric acid (1+9) was added to the acetphenetidin-caffeine residue in the Erlenmeyer flask before the chloroform was boiled off. It was found that the presence of the acid materially aided volatilization of the organic solvent. The caffeine residue was weighed without further purification.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937).

TABLE 1.—*Results of analysis of aspirin, acetphenetidin and caffeine in mixture*

SAMPLE WEIGHT		ASPIRIN	ACETPHENETIDIN	CAFFEINE
	grams	*per cent*	*per cent*	*per cent*
(1)	0.3994	47.65 (45.67)*	—	—
(2)	0.4139	47.08 (45.45)*	47.23	2.78
(3)	0.4177	47.95 (45.99)*	—	2.56
(4)	0.4232	46.68 (45.75)*	—	2.67
(5)	0.4331	47.50 (45.79)*	46.43	2.49
(6)	0.4662	47.00	46.74	2.83
(7)	0.5062	—	—	2.78
(8)	0.5541	47.45	47.14	2.94
(9)	1.0103	46.97	47.46	2.77
(10)	1.0160	46.61	47.12	2.59
(11)	1.4592	—	47.46	3.04
(12)	1.5336	46.91	—	2.77

* Double titration, 1 cc. of 0.1 *N* alkali =0.009 gram of aspirin

DISCUSSION

The results (Table 1) show clearly that little choice exists between mixtures containing up to 0.7 gram of acetphenetidin or as little as 0.2 gram insofar as caffeine recovery is concerned. In the case of Sample 11 the maximum figure for caffeine is associated with a 100 per cent recovery figure for acetphenetidin, thus excluding the possibility that this substance might be represented in the caffeine residue. However, it is of considerable interest to note that without modification of the method, the hydrolysis procedure gives satisfactory results for the relatively large amounts of acetphenetidin taken.

Since three pairs of determinations were run concurrently, it was impractical to extract the aspirin within the one hour limit essential to the use of the double titration method. The aspirin residue was therefore titrated as a simple monobasic acid, the end point with phenolphthalein being quite sharp and definite for one minute, while the pink color still persisted up to the end of three minutes. The extent to which the aspirin hydrolyzed within the approximately two hours of extraction may be seen in the results obtained by double titration of some of the residues. The recovery of aspirin, by the direct titration (1 cc. 0.1 *N* alkali = 0.0180 gram) was from 98.4 to 101 per cent, the mean recovery being 99.6 per cent for the ten determinations. The recovery of acetphenetidin ranged from 98.4 to 100 per cent, with a mean recovery of 99.4 per cent for the seven determinations. The recovery of anhydrous caffeine ranged from 100 to 122 per cent, with a mean recovery of 110 per cent for eleven determinations.

It is recommended that the topic be continued for further work on caffeine in this mixture.

REPORT ON PYRIDIUM

By HARRY J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

The Associate Referee for 1934 recommended a method for the determination of pyridium, *This Journal*, **18**, 551 (1935), that involved extraction of the pyridium base from ammoniacal solution with chloroform, evaporation of the chloroform, and drying of the residue at 100°. The recommendation was not concurred in by the Referee on Drugs, *Ibid.*, 514, because he believed that the Associate Referee's experiments showed pyridium base to be too volatile at 100° for the method to be accurate.

For this reason the present Associate Referee has not further studied this extraction method, which in any case is not specific and is not applicable to pyridium ointment. In considering the possibility of developing some other method for the determination of this compound, it occurred to the Associate Referee that, as pyridium is an azo dye, it might be possible to titrate it with titanous chloride. Experiments to this end showed in fact that hot aqueous solutions of pyridium containing sodium acid tartrate could be titrated sharply with titanous chloride, particularly when a little light green S F yellowish was used as an indicator. Considerable study was then devoted to the adaptation of this method to the determination of pyridium in ointments. This has not yet been carried to a successful conclusion.

Pyridium is nearly insoluble in fats and fat solvents, and fairly readily soluble in hot water, so a simple extraction with hot water will remove most of the pyridium from an ointment. While these relative solubilities hold for pyridium itself (*i.e.*, the monohydrochloride of pyridium base), the free base is much more soluble in ether and fats than it is in water. Consequently, due to hydrolysis of the pyridium, neither water nor very dilute acids will entirely remove the drug from an ointment with any reasonable number of washings. Complete extraction of color from an ointment was most quickly obtained by dissolving the ointment in ether and extracting alternately with water and hydrochloric acid (1+1). This strength acid served to suppress hydrolysis, but it also caused some precipitation of the insoluble dihydrochloride of pyridium base, which the water removed. The combined acid extracts could be evaporated to dryness to remove hydrochloric acid, redissolved in water, and titrated in the same manner as pyridium itself. Duplicate determinations were reasonably close, but recoveries were only about 87 per cent of the amount added. Further study is needed to explain the reason for this shortage, and to devise means for a complete recovery, before the method can be used for the successful assay of pyridium in ointments. There is some uncertain evidence that the trouble may be partly due to the presence of

dyes other than phenylazo-α-α-diaminopyridine in commercial pyridium.

Because the study of the method could not be completed, no samples were sent to collaborators for assay, and the details of the method are not given at this time. It is believed that the method should not be recommended for adoption until it can be applied to all commercial preparations of pyridium, and then only when satisfactory results have been obtained in collaborative study.

It is recommended[1] that the determination of pyridium by titration with titanous chloride be studied further, particularly with regard to the application of this method to the determination of pyridium in pyridium ointment.

REPORT ON AMINOPHYLLINE

By L. E. WARREN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Aminophylline is a compound of theophylline and ethylene-diamine in approximately equimolecular proportions. Because of its increasing importance in therapy, the Associate Referee became interested several years ago in the assay of aminophylline for its theophylline content. Before undertaking the study of the assay of aminophylline it seemed wise to examine the literature concerning the determinations of theophylline.

HISTORICAL

Theophylline has been known for more than half a century as a minor constituent of tea.[2] It was synthesized by Ach[3] and by Traube[4] and the alkaloid now used in medicine is prepared synthetically.

Theophylline has found extensive use in therapeutics—in earlier years as a diuretic and later in certain distressing forms of heart disease. Because of its greater diuretic power and its readier solubilities it is preferred to theobromine by many clinicians. It is soluble in about 140 parts of water, 100 of alcohol, 250 of acetone, and 2000 of petroleum benzin. It is also soluble in dilute solutions of the alkalies and it forms soluble compounds with the salts of many organic acids. Its compound with ethylenediamine, very soluble in water, was sold as a diuretic under the name "Euphyllin"[5] for many years. In 1930 Schmitt[6] suggested the name "Aminophylline" for this compound, and this has been included as a synonym in the U.S.P. XI.[7]

The earliest method found for the assay of theophylline was recorded by Kockum,[8] who dissolved the alkaloid or its salt in warm dilute sodium

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937).
[2] Kossell, *Ber.*, **21**, 2164 (1888); *Z. physiol. Chem.*, **13**, 298 (1889).
[3] *Ber.*, **28**, 3135 (1895).
[4] *Ibid.*, **33**, 3035 (1900).
[5] Dessauer; *Therap. Monatsh.*, **22**, 401 (1908).
[6] *J. Am. Pharm. Assoc.*, **19**, 821 (1930).
[7] Pharmacopoeia of the United States, **11**, 384 (1935).
[8] *Svensk Farm. Tid.*, **12**, 81 (1908); *Apoth. Ztg.*, **23**, 230 (1908).

hydroxide or warm water and titrated the solution with 0.1 *N* silver nitrate, using potassium chromate solution as indicator.

Puckner and Warren[1] used this method with success in 1912. Emery and Spencer[2] published an iodometric assay for theobromine in 1918. They stated that their method was applicable to theophylline and that their results with the method on this alkaloid would be published later. The writer has been unable to find any subsequent publication on the subject by them.

Bruekeleveen[3] modified the Emery-Spencer method for theobromine by substituting normal sodium hydroxide for glacial acetic acid as the initial solvent. He obtained good results with theobromine but he did not attempt to apply his method to theophylline.

Schmitt[4] used an assay procedure by which the theophylline was determined volumetrically with an empiric solution of silver chloride dissolved in ammonium hydroxide.

Self and Rankin[5] published a method for the assay of theophylline and theobromine. This consists in dissolving the material in dilute sodium hydroxide solution and methylating the theophylline to caffeine with dimethyl sulfate in presence of a slight excess of the alkali. The resulting caffeine is then determined by shaking out with chloroform in the usual way. The result is calculated to theophylline by a factor. The results with knowns were about 1.7 per cent low, possibly due to the formation of some substance other than caffeine, which is but slightly soluble.

Ferrey[6] employed the Emery-Spencer method for theobromine for the determination of theophylline in theophylline sodium acetate. Controls with known quantities of theophylline were satisfactory (100.1–100.3 per cent).

Gulland and Macrae[7] state that theophylline forms a definite, scantily soluble complex compound with palladous chloride. They record analytical results for the determination of caffeine and theobromine by the palladous chloride method but give none for theophylline.

The Chemical Laboratory of the American Medical Association[8] published a method for the assay of theophylline in aminophylline. This is the Emery-Spencer iodometric method, modified to omit the solution of the substance in glacial acetic acid. This method did not give satisfactory results.

In 1935 Reimers[9] reported that the argentometric method of Kockum gave high results and that the iodometric process gave low results. He found the Self-Rankin procedure satisfactory but he devised a simpler

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[1] *Rept. Chem. Lab., Am Med. Assoc.*, 3, 119 (1910).
[2] *J. Ind. Eng. Chem*, 10, 605 (1918).
[3] *Chem. Weekblad*, 24, 206 (1927).
[4] *J. Am. Pharm. Assoc.*, 19, 82 (1930).
[5] *Quar. J. Pharm Pharmacol.*, 4, 346 (1931).
[6] *Ibid.*, 340.
[7] *J. Chem. Soc.*, II, 2231 (1932).
[8] *J. Am. Med. Assoc.*, 101, 1314 (1933).
[9] *Dansk Tids. Farm.*, 9, 11 (1935).

one. The alkaloid is liberated with hydrochloric acid after which it is shaken out with a mixture of three volumes of chloroform and one volume of isopropyl alcohol. The solution is washed, the solvent evaporated, and the alkaloid weighed.

EXPERIMENTAL

At the time these studies were begun, no method for the assay of theophylline had been adopted by the A.O.A.C. or by the U. S. Pharmacopoeia. Therefore it seemed desirable, before attempting to assay aminophylline for its theophylline content, to try some of the methods described in the literature on an authentic specimen of theophylline. Accordingly, a commercial specimen was recrystallized from water. It contained 9.14 per cent of water of hydration (theory 9.09 per cent) and 28.27 per cent of nitrogen (theory 28.05 per cent). In other respects it responded to tests for identity and purity of theophylline. Another specimen was prepared by recrystallization from water. The water of hydration was determined, but the nitrogen was not determined as a test for purity.

After an examination of the methods described in the literature, three were selected for trial as the most likely to yield satisfactory results: (I) the Emery-Spencer, (II) the Self-Rankin, and (III) the Reimers.

The products were first assayed by various modifications of the Emery-Spencer method for theobromine, including that suggested by Bruekeleveen. The results are given in Table 1.

In general the results were low and variable. In other words, the excellent results obtained by Ferrey could not be duplicated. The findings confirmed the report by Reimers that the method gives low results. Accordingly, the method was abandoned.

The next procedure tried was the Bruekeleveen modification of the Emery-Spencer assay. These results are included in Table 1. They are better than those obtained with the Emery-Spencer method but are not considered satisfactory.

The next method tried was the Self-Rankin process, which has been adopted by the British Pharmacopoeia[1]. As used, this method is essentially as follows:

Method II, Self-Rankin Procedure

Weigh 0.5–0.6 gram of theophylline into a separator, add 10 cc. of 0.5 *N* NaOH, and shake until the powder is dissolved. Add 1 drop of methyl red indicator (or a strip of litmus paper) and 0.8 cc. of dimethyl sulfate. Shake at intervals of 5 minutes for 30 minutes, adding more 0.5 *N* NaOH if necessary to keep the reaction alkaline. Add 10 cc. of the NaOH and 5 cc. of water. Shake with successive portions of 20 cc. each of $CHCl_3$ until all the caffeine has been removed. Wash the bulked $CHCl_3$ extract once with 5 cc. of water. Shake the wash water with 5 cc. of $CHCl_3$ and add

[1] British Pharmacopoeia, 1932, p. 432.

the washing to the main bulk of solvent. Evaporate the $CHCl_3$, dry the residue at 80°, and weigh as caffeine.

$$C_8H_{10}O_2N_4 \times 0.9278 = C_7H_8O_2N_4.$$

The results by the Self-Rankin process are also given in Table 1. They are considered promising. The following modification of the Self-Rankin process was also tried.

Modified Self-Rankin Method

Weigh 1 gram of theophylline into a beaker and add 15 cc. of 0.5 *N* NaOH, stirring until the material is dissolved. Add a drop of methyl red T.S. and sufficient 10% HCl dropwise from a buret with agitation to insure acidity. Stir well and allow the mixture to stand in a refrigerator overnight. Collect the precipitated theophylline in a weighed Gooch crucible and wash the precipitate with two portions of 5 cc. each of cold water slightly acidulated with HCl. Dry the crucible and contents at 80° and weigh as anhydrous theophylline.

Unite the filtrate and washings and evaporate to a volume of about 10 cc. Transfer to a separator with a little water. Add 3 cc. of 0.5 *N* NaOH (or sufficient to render the solution distinctly alkaline) and 0.5 cc. of dimethyl sulfate, and shake well. Set aside for an hour, shaking occasionally. If necessary, add a few drops of 0.5 *N* NaOH from time to time to keep the solution alkaline. Add 10 cc. of 0.5 *N* NaOH and 5 cc. of water to destroy the excess of dimethyl sulfate, and shake for 1 minute. Extract the caffeine immediately by shaking with several 30 cc. portions of $CHCl_3$. Wash each successive portion of $CHCl_3$ in a second separator with 10 cc. of 1% HCl. Remove the $CHCl_3$ from the mixed $CHCl_3$ extracts by evaporation, dry the residue of caffeine at 80° for 1 hour, and weigh. Multiply the weight obtained by 0.9278 and add the product to the weight of theophylline previously obtained.

The results by the modified Self-Rankin method were not considered superior to those obtained by the original process. The method was adopted for aminophylline, later by the Committee of Revision for the United States Pharmacopoeia XI, essentially as given here.

The Reimers procedure follows:

Method III, Reimers' Procedure

Weigh 0.3 gram of theophylline into a separator, add 5 cc. of 0.5 *N* NaOH, and shake the mixture gently until the powder is dissolved. Add 2 drops of methyl red T.S.* and sufficient 0.5 *N* HCl from a buret to produce a distinct acid reaction. Then add 0.5 cc. more of the acid. Add 25 cc. of $CHCl_3$-isopropyl alcohol mixture (3+1) and shake for 1 minute. Allow to settle for at least 5 minutes and draw off the lower layer into a second separator that contains 10 cc. of water and a few drops of 0.5 *N* HCl. Shake well, allow to settle at least 5 minutes, and filter the solvent into a weighed flask through a pledget of cotton placed in the stem of a funnel. Repeat the extraction with five more portions of 20 cc. each of the $CHCl_3$-isopropyl alcohol mixture, wash each portion through the second separator, and pass the solvent through the filter into the weighed flask. (Insure complete extraction by a seventh shaking with 10 cc. of the solvent and evaporation of the solvent in a separate container.) Recover most of the solvent and evaporate the remainder on the steam bath while rotating the container in an inclined position. Add 2 cc. of absolute ether

* At the suggestion of Collaborator Glycart the indicator was later changed to litmus paper.

TABLE 1.—*Results of analyses of theophylline by several methods*

METHOD	LOT	WATER (LOSS AT 80°)	THEOPHYLLINE ANHYDROUS	TOTAL
		per cent	*per cent*	*per cent*
Emery-Spencer	I	9.14 (average)	81.12 75.91	90.27 85.05
	II	7.49 7.95 7.78	85.73 85.78 85.68	93.22 93.73 93.46
Bruekeleveen's modification of Emery-Spencer method	I	9.14 (average)	89.04 90.32	98 18 99.46
	II	7.74 (average)	90.77	98.91
Self-Rankin	I	9.14 9.15	90.43 91.15	99.57 100.30
	I(a)	8 89	93.96	102.85
	II	7.74 (average)	93.10	100 84
Self-Rankin Modified	I	9.14 (average)	90.84	99.98
	I(a)	8.27 8.89	89 91 90.70	98 18 99.59
	II(a)	6 51	92 90	99.41
	III(a)	7.29	90.76	98.05
Reimers	II	7.74 (average)	92.80 92 68 92.42	100 45 100.42 100.16
Reimers (Automatic)	I(a)	9.08 8.87 8.64	91.69 94.09 94.70	100.77 102 96 103.34
	I(c)	8.915 8.915	92.65 92.70	101.57 101.62

to the residue, evaporate (cautiously to avoid spattering), and dry the residue at 80°. Weigh as anhydrous theophylline.

$$C_7H_8O_2N_4 \times 1.100 = C_7H_8O_2N_4 + H_2O.$$

The findings by the Reimers process are given in Table 1. They are

considered sufficiently satisfactory to warrant collaborative study of the method.

Since the shake-out method with chloroform-isopropyl alcohol solvent gave good results it seemed worth while to try the same solvent in a continuous extraction apparatus. Because the ingredients of the solvent mixture do not have identical boiling points it seemed best to try the extractions in the presence of a 2/3 saturated solution of NaCl. After several trials the following procedure was worked out:

Charge a continuous extraction apparatus (for liquids heavier than water) with a mixture of $CHCl_3$ and isopropyl alcohol (3+1). Weigh 0.2–0.3 gram of theophylline (or a quantity of material containing an equivalent amount of alkaloid) into a beaker, add 10 cc. of 0.5 *N* NaOH, and shake the mixture gently until the alkaloid is dissolved.

Transfer the solution (or mixture) to the extraction apparatus, using small quantities of water for washing, and add 20 cc. of saturated salt solution. Add a strip of litmus paper and sufficient 10% HCl to produce a distinct acid reaction. Extract with the $CHCl_3$-isopropyl alcohol mixture for 3 hours, or until the solution is exhausted of theophylline. Transfer the extract to a separator that contains 10 cc. of water and a few drops of 5% HCl. Shake for 1 minute and allow to settle until clear. Repeat the washing with acidified water in a second separator. Filter the solvent into a weighed flask through a pledget of cotton placed in the stem of a funnel. Recover most of the solvent and evaporate the remainder on the steam bath while rotating the container in an inclined position. Add 2 cc. of absolute ether to the residue and evaporate cautiously (to avoid spattering). Dry the residue at 80° to constant weight and weigh as anhydrous theophylline.

$$C_7H_8O_2N_4 \times 1.100 = C_7H_8O_2N_4 + H_2O.$$

The results are given in Table 1.

To simulate aminophylline, several mixtures were prepared containing known quantities of anhydrous theophylline, together with an excess of ethylenediamine. These were analyzed for theophylline by the Reimers process and by the Self-Rankin procedure. The findings are given in Table 2. The results by the Self-Rankin procedure are high.

TABLE 2.—*Results of analyses of theophylline-ethylenediamine mixtures by the Reimers and the Self-Rankin procedures*

METHOD	COMPOSITION OF MIXTURES		THEOPHYLLINE RECOVERED (ANHYDROUS)	
	THEOPHYLLINE TAKEN (ANHYDROUS)	ETHYLENEDIAMINE TAKEN		
	gram	*gram*	*gram*	*per cent*
Reimers	0.2820	0.1956	0.2838	100.64
	0.5807	0.2129	0.5771	99.38
Self-Rankin	0.5665	0.1825	0.5845	103.33
	0.5881	0.2011	0.6125	104.20

The modified Self-Rankin method was next tried on a commercial

specimen of aminophylline. Low results were obtained in the preliminary trials. Apparently a larger quantity of dimethyl sulfate must be used in the assay of aminophylline than with most other theophylline preparations, probably because ethylenediamine combines with the reagent. The results from several trials (by the use of sufficient reagent) are given in Table 3.

TABLE 3.—*Results of assays of aminophylline by Self-Rankin modified method*

EXPERIMENT	THEOPHYLLINE BY PRECIPITATION	THEOPHYLLINE BY METHYLATION	TOTAL THEOPHYLLINE (ANHYDROUS)
	per cent	*per cent*	*per cent*
A	65.74	4.18	69.92
B	66.76	3.14	69.90
C	67.45	1.65	69.10
D	69.10	1.09	70.19
E	68.16	1.92	70.08
Average	67.44	2.40	69.82

The results for theophylline (Table 3) are reasonably consistent although the actual theophylline content of the preparation was not known.

COLLABORATIVE STUDIES

Two specimens of theophylline and two of a mixture of theophylline and starch were sent to collaborators with a request for analysis by the Self-Rankin method and by the Reimers process. The calculated composition of these products is given in Table 4. The details of the process are as follows:

Method IV, Theophylline and Starch

Weigh 0.5 gram of the material into a separator, add 5 cc. of 0.5 *N* NaOH, and shake the mixture gently for about 5 minutes. Add 2 drops of methyl red indicator (or a strip of litmus paper), and complete the assay as directed in Method III.

TABLE 4.—*Calculated composition of theophylline mixtures*

SPECIMEN	WATER (LOSS AT 80°)*	THEOPHYLLINE ANHYDROUS (BY DIFFERENCE)	STARCH (AIR-DRIED)
	per cent	*per cent*	*per cent*
Theophylline Lot IC	8.915	91.085	—
Theophylline and Starch	4.384	44.790	50.826
Theophylline Lot IIC	9.242	90.758	—
Theophylline and Starch Lot II	5.545	54 455	40.000

* Calculated from the theophylline content

The results obtained by the collaborators are given in Table 5.

Three specimens of aminophylline preparations were prepared for collaborative study: (1) commercial aminophylline; (2) 50 per cent of aminophylline and known amounts of starch, acacia, and talc; (3)

TABLE 5.—*Results of analyses of theophylline preparations by several collaborators*

SPECIMEN	COLLABORATORS	THEOPHYLLINE (ANHYDROUS)	THEOPHYLLINE (CALCULATED TO $T+H_2O$)	THEORETICAL COMPOSITION		RECOVERY (AVERAGE)
				LOSS AT 80°	THEOPHYLLINE BY DIFFERENCE	
		Method II, Self-Rankin Procedure				
		per cent	*per cent*			
	P. S. Jorgensen	86.64 88.74	95.30			95.61
			97.61			
	C. K. Glycart	90.42	99.46			97.42
Theophylline	L. E. Warren	87.32 88.83	96.05 97.71			97.04
Lot IIC	F. C. Sinton	Method unsatisfactory		9.242	90.758	
	S. W. Bower	88.84	97.92			
		91.66	100.83			99.44
	P. S. Jorgensen	52.55 52.05	57.47 57.26			96.06
	C. K. Glycart	53.48	58.83			98.20
Theophylline	L. E. Warren	51.45	56.60			96.11 97.74
and Starch		53.32	58.65			
Lot II	F. C. Sinton	Method unsatisfactory			54.45	
	S. W. Bower	50.79	55.87			93.27
		Method III, Reimers' Procedure				
	P. S. Jorgensen	91.16	100.28			
		91.16	100.28			
		91.33	100.47			100.14
Theophylline	C. K. Glycart	91.27	100.4	8.915	91.085	100.20
Lot IC	L. E. Warren	91.40 90.95	100.54 100.04			
		91.67 91.72	100.84 100.89			100.38
	S. W. Bower	91.90 92.00	101.09 101.20			
		90.70	99.77			100.68

SPECIMEN	COLLABORATORS	THEOPHYLLINE (ANHYDROUS)	THEOPHYLLINE (CALCULATED TO T+H_2O)	THEORETICAL COMPOSITION		RECOVERY (AVERAGE)
				LOSS AT 80°	THEOPHYLLINE BY DIFFERENCE	
		per cent	*per cent*			
Theophylline Lot IIC	P. S. Jorgensen	92.99 93.08	102.29			102.51
			102.39			
	C. K. Glycart	91.01	100.11	9.242	90.758	100.28
	L. E. Warren	95.86	105.45*			105.62*
		93.98 93.33	103.38 102.66			103.19
	F. C. Sinton	91.50	100 65			100.41
		91.50	100.65			
	S. W. Bower	91.20	100.32			
		91.00	100.08			100.20
Theophylline and Starch Lot I	P. S. Jorgensen	44.80 44.70	49.28 49.17		(By calculation)	
		44.70	49 17			100.15
	C. K. Glycart	44.36	48 80			99.04
	L. E. Warren	44 50	48 95		44.79	99.42
		44.56	49.02			
	S. W. Bower	44.38 44.50	48 82 48 94			99 09
		44 26	48.68			
Theophylline and Starch Lot II	P. S. Jorgensen	55.14 54 65	60 65 60.11			100 88
	C. K. Glycart	54.83	60 32			100.70
	L. E. Warren	55 38	60 91 61.21			
		55.65			54.455	101.96
	F. C. Sinton	55.0	60.50			101.3
		55.3	60.83			
	S. W. Bower	54.76 55.18	60 24 60.70			
		54 92	60.40			100.91

* The analyst is unable to account for this very high result.

marketed tablets of aminophylline that had been pulverized and sieved. Subdivisions were sent to each of five collaborators with the request that the material be assayed by the Reimers process, modified if found necessary by the excipients. The detailed directions follow:

Method IV, Aminophylline

Weigh 0.3 gram of the material into a separator, add 5 cc. of water, and shake gently until solution takes place. Add 2 drops of methyl red indicator, or a strip of litmus paper and complete the assay as directed in Method III.

Method V, Aminophylline and Starch

Weigh 0.8 gram of the material into a small beaker, add 5 cc. of water, stir thoroughly, and allow to settle. Decant the supernatant liquid through a small filter into a separator. Repeat the maceration, decantation, and filtration 4 times more, each with 5 cc. of water. Wash the beaker and filter with 5 cc. more of water. Add 2 drops of methyl red indicator (or a strip of litmus paper) to the united filtrate and washing and complete the assay as directed in Method III.

Method VI, Aminophylline Tablets

Use Method V. The findings are given in Table 6.

TABLE 6.—*Results of analyses of aminophylline preparations by several collaborators*
Method III, Reimers' Procedure

SPECIMEN	COLLABORATORS	THEOPHYLLINE		THEOPHYLLINE CALCULATED TO $T+H_2O$	
		per cent		*per cent*	
Aminophylline	P. S. Jorgensen	80.83	80.67	88.91	88.73
		80.83		88.91	
	C. K. Glycart	79.64		87.60	
	L. E. Warren	80.76	80.79	88.84	88.87
	S. W. Bower	80.60	80.50	88.66	88.55
		80.67		88.74	
Aminophylline and Starch	P. S. Jorgensen	40.87	40.87	44.96	44.96
		40.93		45.03	
	C. K. Glycart	40.25		44.28	
	L. E. Warren	40.53	40.84	44.58	44.92
	S. W. Bower	41.71	41.36	45.88	45.50
Aminophylline Tablets	P. S. Jorgensen	35.62	35.75	39.19	39.32
		35.75		39.32	
	C. K. Glycart	35.00		38.50	
	L. E. Warren	35.76	36.02	39.34	39.62
	S. W. Bower	36.35	35.95	39.99	39.55

COMMENTS BY COLLABORATORS

P. S. Jorgensen.—It is possible to improve the method for aminophylline and starch by simplifying the technic. Instead of dissolving the sample by maceration and decantation with several portions of water, the sample may be added directly to the separator as directed in Method III and dissolved in a small volume of water by shaking several minutes. It seems advantageous to keep the volume of water as small as possible at this point because if large volumes are used more washings with

immiscible solvent are necessary to effect complete extraction (Sample IV). Aminophylline and starch assayed in this manner gave 40.84% anhydrous theophylline or 44.92% crystalline theophylline. This checks favorably with the results obtained by the other method. It was also noted that the dimethyl sulfate reagent had attacked the cork and had become discolored. This might account for the lower results by this method.

C. K. Glycart.—The dimethyl sulfate received from you had turned black. Consequently I used our reagent, which had been recently purchased. I suggest that a small piece of litmus paper be added, instead of methyl red as directed since the residue of theophylline contained methyl red. Suggest that the phrase "to constant weight" be added to the directions because the anhydrous theophylline requires rapid weighing. Suggest seven extractions instead of five as directed since corrections for incomplete extractions were required for each sample.

F. C. Sinton.—The method appears to be satisfactory, and no difficulty was encountered. I made several attempts to follow Method II, using dimethyl sulfate reagent, without success. I found that it was difficult to get complete extraction, and that even after about ten extractions the weight of extract was only about one-half that obtained by Method I. The difficulty was likely due to decomposition of the dimethyl sulfate. The reagent was highly colored and the cork stopper showed signs of considerable decomposition. It appears, therefore, that by using the decomposed reagent only a limited amount of methylation was obtained.

S. W. Bower.—The dimethyl sulfate furnished showed discoloration from the cork so a new supply was obtained. I spent some time in experimenting with the Self-Rankin method. Inconsistent results were obtained. For example, with 0.5 gram of sample and 0.5 cc. of dimethyl sulfate 0.2737 gram of anhydrous caffeine was obtained, but with 0.5 gram of sample and 0.8 cc. of the reagent the weights of residue as anhydrous caffeine were 0.3382 and 0.3334 gram. This indicates the formation of some other compound than caffeine.

L. E. Warren.—In shaking out tablet mixtures in alkaline solution (Self-Rankin process) for the first time, troublesome emulsions may form. These will usually clear up on standing a few minutes. After the first shake-out, they cease to give trouble. In general I found the Self-Rankin process less satisfactory than the Reimers.

CONCLUSIONS

(1) The Emery-Spencer method for the determination of theobromine gives low and erratic results for theophylline.

(2) The Bruekeleveen modification of the Emery-Spencer method is moderately satisfactory.

(3) The Self-Rankin process gives results that are about 3 per cent low; occasionally the results are erratic. Possibly the method is not so satisfactory for theophylline as for theobromine. The method is unsatisfactory in the hands of some analysts.

(4) The Reimers process is the most satisfactory of any tried. The results tend to be high, usually 0.25–0.6 per cent above theory, although the excess may amount to 3 per cent.

It is recommended[1] that the method for theophylline that was subjected to collaborative study be adopted as tentative, *This Journal*, **20**, 82 (1937), and that further study of aminophylline be dropped because the U. S. Pharmacopoeia has adopted a method of assay.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 56 (1937).

REPORT ON GUMS IN DRUGS

By J. H. CANNON* (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Study of this subject was continued in accordance with the recommendations made last year. As indicated in the Referee's report last year, *This Journal* **19**, 528 (1936), it was found that when separated from drug mixtures certain gums fail to yield the reactions observed in pure gum suspensions in water. This point led to the present study of methods which would more certainly establish the identity of the gum in question.

Of the several procedures that were tried, the most promising is a microscopical examination of the precipitate obtained with alcohol. The special microscope accessories found to be most useful are the polarizer and the dark field illuminator. Reagents used were limited to 95 per cent alcohol saturated with sodium chloride, tincture of iodine, and zinc-chlor-iodine solution (10 grams of KI and 0.1 gram of iodine dissolved in 100 cc. of 60 per cent $ZnCl_2$ solution). The general procedure is as follows:

To a concentrated aqueous gum suspension add 4 volumes of the alcohol-NaCl solution, mix, and centrifuge. Decant the alcohol from the compacted gum precipitate and cover it with 95 per cent alcohol. Let stand for an hour to further harden and dehydrate the precipitate. Remove a particle of the solid material to a clean slide and press flat and thin as possible beneath a cover-glass. (Examination at this stage with low power and with dark field illumination will give considerable information as to the type of gum dealt with.

Carefully remove the cover-glass so as to leave the thin mat of precipitate attached to the slide. Cover the gum with a drop of tincture of iodine, allow the alcohol to evaporate, and cover with a drop of zinc-chlor-iodine. Examine in polarized light from time to time during several minutes.

DISCUSSION

The direct examination of the gum with dark field illumination will show two general types of precipitate, one in which a definite stringy structure is present and the other consisting apparently of tiny non-crystalline particles of uniform size and consistency. Of the gums studied, only agar and acacia fall in the latter group.

Tragacanth, quince seed, and Irish moss appear stringy and are not easily confused with acacia and agar. Examination in polarized light before and after treatment with iodine and zinc-chlor-iodine reveals certain structural differences between tragacanth, quince seed, and Irish moss precipitates which apparently can be made the basis of positive identification of these gums.

Study of the above scheme was limited to five or six gums. The data thus far accumulated are not sufficiently comprehensive to warrant collaborative study. It is felt, however, that the possibilities suggested by

* Presented by A. E. Paul.

this procedure are such as to warrant a continuation of the work along similar lines.

It is recommended that the study of gums in drugs be continued. References: Woodruff and Hayden, *J. Agr. Research*, **52**, 233–7 (1936); Sjostrom, *Ind. Eng. Chem.*, **28**, 63–74 (1936).

REPORT ON CINCHOPHEN AND SODIUM BICARBONATE TABLETS

By R. L. VANDAVEER* (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In agreement with the recommendations of Subcommittee B, a method for cinchophen in tablets with sodium bicarbonate was studied collaboratively.

Cinchophen tablets may be assayed by dissolving and titrating the cinchophen in neutral alcohol (N.F. VI procedure). Various analysts have found that bicarbonate of soda, which is often compounded in

COLLABORATOR	WEIGHED AMOUNTS OF—		RECOVERY OF CINCHOPHEN BY TITRATION	
	CINCHOPHEN	$NaHCO_3$		
	gram	*gram*	*gram*	*per cent*
I. S. Shupe St. Louis	0.4061	0.86	0.406	100 0
	0 4138	0 47	0 413	99 8
	0 4051	0.40	0.406	100 2
	0.4261	none	0.426	100.0
E. H. Berry Chicago	0.4000	0.4	0 3986	99 6
	0 4000	0.4	0 3978	99.5
M. Harris Chicago	0 3983	0.3984	0 3973	99 8
	0.4004	0.4009	0.4003	100 0
H. R. Bond Chicago	0.400	0.4	0 398	99 5
	0.400	0.4	0.403	100 8
R. L. Vandaveer Chicago	0.500	0.5	0.5039	100 8
	0.506	0 5	0 5052	99.8

cinchophen tablets, causes erroneous results by this procedure. In the method presented by the Associate Referee the cinchophen is extracted from an acid solution, the solvent is evaporated, and cinchophen is determined by titration with 0.1 *N* alkali.

Best quality cinchophen and sodium bicarbonate were obtained to prepare collaborative samples. It was not feasible to prepare a mixture

* Presented by E. M. Hoshall.

of the two powders since cinchophen agglomerates, and it is almost impossible to obtain a uniform sample. So a subdivision of the cinchophen and sodium bicarbonate was sent to each collaborator with instructions to "accurately weigh 0.4000 gram of cinchophen and 0.4 gram of sodium bicarbonate and transfer to a separator. Then to follow the method for cinchophen in cinchophen and sodium bicarbonate tablets, beginning "add dilute sodium hydroxide to dissolve cinchophen." The method was published in *This Journal*, **20**, 83 (1937).

The collaborative results are given in the table.

DISCUSSION

The results of the analysts are in excellent agreement, and they report no difficulty with the method, except that all used heat to dissolve the cinchophen in the 60 cc. of neutral alcohol. Consequently, the method should be amended to read, "dissolve the residue in 60 cc. of neutral alcohol by warming."

It is recommended[1] that the procedure presented be adopted as a tentative method and that the work on cinchophen and sodium bicarbonate tablets be discontinued.

REPORT ON THE STABILITY OF POTASSIUM IODATE VOLUMETRIC SOLUTIONS

By SOLOMON M. BERMAN (U. S. Food and Drug Administration, Philadelphia, Pa.), *Associate Referee*

Early last year directions for preparing and standardizing a 0.025 *M* solution of potassium iodate were submitted to several laboratories. The purpose was to test the keeping quality of such a solution over a prolonged period, such as two years. The standardizations were made at intervals of approximately 3 months, and as finally reported they cover a period of 20 months. The results are given in Table 1.

COMMENTS OF COLLABORATORS

W. F. Reindollar.—Two liters of 0.025 *M* solution were prepared from Merck's reagent potassium iodate. The solution was preserved in a glass-stoppered, 2 liter flask in a dark cupboard. Bureau of Standards arsenious oxide was employed as the standardizing agent. On several days on which the determinations were run the air was very humid, making the weighings rather difficult. I do not believe that the solution has changed substantially since it was prepared.

C. F. Bruening.—The solution was prepared from potassium iodate C P, manufactured by the J. T. Baker Chemical Co. A slight turbidity in the prepared solution was filtered off before the initial standardization. The solution was kept in a glass-stoppered Pyrex bottle in a dust-free cabinet with glass doors that allowed diffused light to enter. After 3 months a small amount of stringy mold formation was noticed, but it did not increase during the remaining time of the experiment. The arsenious oxide used was Bureau of Standards Sample No. 83.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 57 (1937).

TABLE 1.—*Standardization of 0.025 M potassium iodate solution by As_2O_3**

COLLABORATOR	MARCH 1935	JUNE 1935	SEPT. 1935	DEC. 1935	MARCH 1936	JUNE 1936	SEPT. 1936	NOV. 1936
W. Reindollar	1.000	0.997	0.997	0.994	1.003	1.003	0.994	
	0.999	1.001	0.992	1.000	0.994	1.003	1.005	
C. F. Bruening	1.002		1.003	1.000			1.000	
	1.002		1.001	1.001			1.003	
H. O. Moraw	1.000	0.999	1.003					
	1.000	1.000	0.999					
S. M. Berman	0.998	0.999	1.001				1.005	1.006
	1.000	1.002	1.002				1.006	1.004
	1.002							1.006

* Note: When factors were reported to four decimals, only three were retained, since the precision involved is at best one part per thousand.

H. O. Moraw.—Two liters were prepared from Eimer and Amend C P potassium iodate and stored in a glass-stoppered acid bottle kept in a dark cupboard.

Associate Referee.—The potassium iodate used was C P grade supplied by Bausch and Lomb perhaps 20 years ago. The solution was kept in a glass-stoppered acid bottle in a dark cupboard. In April 1936, the bottle was stored for several days in a cupboard that was found to attain a temperature of about 40°C. due to the rays of the early morning sun. The subsequent standardizations may be high because of some evaporation of water in the period mentioned. After a few months the solution acquired a slight amount of sediment.

DISCUSSION OF RESULTS

The factors reported by the collaborators are in excellent agreement, considering that the weight of primary standard taken permits the use of no more than four significant figures at any stage. For the sake of precision a larger weight might have been dissolved in a suitable volume of solution, and aliquots taken for the titrations, but the factors as actually obtained are probably more informative. In only one case were the factors consistently above 1.000, and here the solution had been filtered, with the likelihood that the solution was slightly concentrated by evaporation. While it appears that the potassium iodate available from different sources is sufficiently pure to be used as a standard, and that the solutions prepared therefrom are of satisfactory stability, it is desirable to have a convenient chemical method for calibrating the solution. The method supplied to the collaborators is suitable for this purpose.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method of preparing standard solutions of potassium iodate by dissolving a calculated weight of the salt in a definite volume of water, *This Journal*, **19**, 537 (1936), be adopted as tentative.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 57 (1937).

(2) That the proposed method for standardizing potassium iodate solutions be adopted as tentative.

(3) That the topic be closed.

REPORT ON DINITROPHENOL

By W. F. KUNKE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The study of the quantitative determination of alpha, or 2–4 dinitrophenol, singly or in admixture, was continued in accordance with the recommendation of Subcommittee B. Last year a partial critical investigation of the bromination of this dinitrophenol was made and it appeared desirable to continue the study and to develop a method for the extraction from a mixture, such as usually found in tablets and capsules, before submitting the methods to collaborative study.

The results of the critical experimental and collaborative study with the methods devised and recommendations are included in this report. No method or experimental work had been reported to this Association previously. Alpha, or 2–4 dinitrophenol, and its sodium compound have been used recently as metabolic stimulants[1] and as an ingredient in obesity preparations.

REVIEW OF LITERATURE

Alpha dinitrophenol has a strong acid character. Suchier[2] took advantage of this in his quantitative method by titrating the aqueous solution of dinitrophenol with 0.5 *N* sodium hydroxide, using phenolphthalein as indicator. This method was not tried. Shupe, *This Journal*, **18**, 464 (1935), reported a procedure for the detection and determination of alpha, or 2–4 dinitrophenol, in a mixture. Day and Taggert[3] worked out a general analytical procedure for the Koppeschaar method, which they reported is applicable, with a few modifications, to a large number of compounds including 2–4 dinitrophenol.

The bromination method for phenol, first developed by Koppeschaar,[4] has been used for the quantitative determination of a number of phenols and aromatic amines. Redmen, Weith, and Brock,[5] made the first critical and conclusive investigation of the Koppeschaar method for phenol. Day and Taggart reported good results for salicylic acid at high acid concentration but Kolthoff[6] states that this is not correct. Kolthoff found that if more than 5 cc. of 4 *N* hydrochloric acid (15 per cent) is used per 50 cc. of solution in the analysis of salicylic acid, too little bromine is con-

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[1] *J. Am. Med. Assoc.*, 101, 3, 193 (1933).
[2] *Z. Anal. Chem*, 85, 434 (1931).
[3] *J. Ind. Eng. Chem.*, 20, 545 (1928).
[4] *Z. Anal. Chem.*, 15, 233 (1876).
[5] *J. Ind. Eng. Chem.*, 5, 389 (1913).
[6] Volumetric Analysis, 2, 477 (1929).

sumed, for example when using 10 cc. and 15 cc. of the acid the errors are −8 per cent and −14 per cent, respectively. According to Kolthoff the quantity of excess bromine has only a subordinate influence.

BROMINATION METHOD—CONDITIONS NECESSARY FOR QUANTITATIVE DETERMINATION

Although the excess bromination method or Koppeschaar method is well known and gives good results when properly used, the literature is notably meager with reference to the influence of a variation of the different conditions, namely, acid concentration, quantity of excess bromine, and period of bromination. In order definitely to establish the correct working conditions and their resonable limits for quantitative determination of 2–4 dinitrophenol, the critical experimental study was made. Under proper conditions 2–4 dinitrophenol forms a monobrom compound according to the equation:

$$C_6H_3(OH)(NO_2)_2 + Br_2 = C_6H_3Br(OH)(NO_2)_2 + H\ Br.$$

Table 1 gives the results of the experiments made under the following most favorable conditions for accurate quantitative work: Bromine used from 25 to 100 per cent excess, volume from 90 to 140 cc., acid content from 5 to 10 cc., and bromination period from 1 to 3 minutes. The purified 2-4 dinitrophenol had a melting point of 114°C., and showed no loss in weight when kept over sulfuric acid.

TABLE 1.—*Results obtained under working conditions indicated*

EXP. NO.*	0.1 N BROMIDE-BROMATE ADDED	0.1 N BROMIDE-BROMATE CONSUMED	TOTAL VOLUME†	HCl (CON.)	BROMINATION PERIOD	DINITROPHENOL FOUND
	cc.	*cc.*	*cc.*	*cc.*	*minutes*	*per cent*
1	25	19.97	110	5	1	99.9
2	25	19.98	110	5	1	99.9
3	25	19.96	110	5	1	99.8
4	25	19.93	90	5	1	99.6
5	25	19 95	90	5	1	99.8
6	25	19.97	90	5	1	99.9
7	25	19.93	90	5	1	99.7
8	25	19.90	90	5	1	99.5
9	25	19.90	90	5	1	99.5
10	25	19.92	125	5	1	99.6
11	25	19.98	125	5	1	99.9
12	25	19.92	125	7.5	2	99.6
13	25	19 98	125	7.5	2	99.9
14	25	19.92	125	10	1	99.6
15	25	19.98	125	10	1	99 9
16	25	19.96	125	10	3	99.8
17	25	19.96	125	10	1	99.8
18	40	19.96	140	5	1	99.8

* 20 cc. aliquot containing 0.1841 gram of dinitrophenol (about 0.4% NaOH solution).
† Water used for dilution.

The conditions indicated in Table 1 are embodied in the proposed method. It gives accurate results, particularly when it is taken into consideration that a difference of 0.05 cc. of 0.1 *N* sodium thiosulfate in the back titration would calculate out to a difference of 0.26 per cent in the result. With a larger sample and larger volume of 0.1 *N* bromide-bromate consumed, the difference in result would be correspondingly smaller. Results within so narrow a range as are obtained for phenol by the Koppeschaar method under correct conditions are not to be expected because the bromine consumed per molecular weight of phenol is three times that of 2–4 dinitrophenol.

The procedure of Day and Taggart calls for 100 per cent excess of bromine. This is not necessary for accurate results, although if accompanied with a 1–3 minute bromination period 100 per cent excess of bromine does no harm. However, a 25 per cent excess of bromine or even less is sufficient for accurate results. It may be noted that the larger the quantity of unconsumed bromine the greater the chance of loss with its attendant error.

Table 2 gives the results of experiments to show the effect, if any, on the result, of a change in acid concentration, quantity of excess bromine, or bromination period from those indicated in Table 1.

TABLE 2.—*Results obtained under unfavorable conditions*

EXP. NO.	0.1 *N* BROMIDE-BROMATE		TOTAL VOLUME	HCl (CON.)	BROMINATION PERIOD	DINITROPHENOL FOUND
	ADDED	CONSUMED				
	cc.	*cc.*	*cc*	*cc.*	*minutes*	*per cent*
1	40	20.78	140	5	30	103.9
2	40	20.84	140	5	30	104.2
3	25	20.02	125	10	30	100.1
4	25	20.04	125	10	30	100.2
5	25	20.00	200	10	10	100.0
6	25	20.00	125	10	30	100.0
7	25	20.06	125	5	30	100.3
8	25	20.18	125	5	30	100.9
9	20	19.42	125	5	1	97.1
10	20	19.72	125	5	1	98.6
11	25	15.52	125	3	1	77.6
12	25	19.76	125	3	1	98.8
13	40	15.3	140	2	1	76.5
14	25	11.46	125	2	2	57.3
15	25	4.18	125	1	2	20.9
16	40	13.22	140	1	1	66.1
17	40	13.25	140	1	1	66.2
18	40	11.72	140	1	30	58.6
19	40	7.5	120	1	3	37.5
20	20	5.24	120	1	1	26.8
21	20	3.84	120	1	1	19.2

The results (Table 2) show definitely that—

(1) A 30 minute bromination period with about 100 per cent excess bromine gives an error of about +4 per cent (Exp. Nos. 1 and 2).

(2) A 30 minute bromination period with about 25 per cent excess bromine only occasionally gives an error of about +0.9 per cent (Exp. Nos. 3–8, inclusive).

(3) Bromine sufficient for the theoretical requirement, without an excess, may give an error of about −3 per cent (Exp. Nos. 9–10).

(4) An acid concentration of less than 5 cc. of concentrated hydrochloric acid per 125 cc. of liquid gives an error of as much as −80 per cent.

In short, the results show that an accurate quantitative method should be limited to the working conditions indicated in Table 1.

EXTRACTION OF 2–4 DINITROPHENOL

One gram of 2–4 dinitrophenol was found to be soluble in not less than 3000 cc. of water at room temperature (22°C.) and in about 9 cc. of chloroform. Dinitrophenol readily reacts with sodium hydroxide to form the sodium compound. The sodium compound is very soluble in water (1 gram in about 22 cc.) but insoluble in chloroform. Accordingly, it may be expected that this dinitrophenol is readily extracted from aqueous mixture by chloroform or from chloroform solution by dilute sodium hydroxide solution. These characteristics are the basis of the proposed method for mixtures.

It was found that dinitrophenol forms some emulsion during extraction with chloroform from an acidified aqueous mixture of starch, lactose, and calcium carbonate, particularly if vigorously shaken during the first two extractions. A larger volume of chloroform or acid-aqueous mixture with less vigorous shaking prevents a troublesome emulsion. If thyroid is present in the above mixture, the emulsion is very troublesome.

A mixture containing 18.4 per cent of 2–4 dinitrophenol was prepared by thoroughly mixing equal parts by weight of starch, lactose, and calcium carbonate with the dinitrophenol. Some or all of these ingredients may be expected in a tablet or capsule.

Determinations were made by the proposed method, and the results obtained were 18.3, 18.35 and 18.4 per cent.

Alpha or 2–4 dinitrophenol sublimes slowly at the temperature of a steam bath; for example, when a beaker containing 0.2000 gram was exposed to steam the loss in weight in 30 minutes was 4.4 per cent, and in 100 minutes it was 14.4 per cent. The dinitrophenol may be extracted, the last 5 cc. of chloroform evaporated spontaneously and weighed with accurate results, and this procedure may be used as an alternative method. Determinations made on the mixture containing 18.4 per cent 2–4 dinitrophenol by this alternative method yielded 18.42 and 18.44 per cent of 2–4 dinitrophenol. This method was not submitted for collaborative study.

EXPLOSIVE PROPERTIES

The literature warns against grinding or heating 2–4 dinitrophenol or its salts because of danger of an explosion. However, many coated and uncoated tablets of 2–4 dinitrophenol or its sodium compound containing various ingredients were ground in a mortar to a uniform powder and no explosion of any kind occurred. Alpha, or 2–4 dinitrophenol, and the sodium salt, in mixtures and tablets, were subjected to violent blows with a hammer and there was no indication of explosion. This dinitrophenol was heated on a steam bath (without an air current) and no explosion resulted. Bird and co-workers[1] reported that alpha dinitrophenol melts and sublimes unchanged when heated gently. When slowly heated in an open crucible over a flame the material catches fire and burns quietly.

Bird determined the water of crystallization of sodium dinitrophenate by heating for 4 hours at 160°–170°C. However, during careful attempts to determine the sodium content by simple ignition, explosions resulted. This is characteristic of salts of many aromatic nitro compounds, in that the sodium salt is much more sensitive to heat than the parent nitro compound.

PROPOSED METHOD FOR 2–4 DINITROPHENOL OR ITS SODIUM COMPOUND

REAGENTS

(a) *Sodium hydroxide solution.*—Dissolve 2 grams of NaOH in 100 cc. of water.

(b) *Hydrochloric acid.*—35–37 per cent.

(c) *Potassium iodide solution.*—Dissolve 20 grams of KI in 100 cc. of water.

(d) *Bromide-bromate solution.*—0.1 *N*. Dissolve 2.7835 grams of $KBrO_3$ or its equivalent and 12 grams of KBr in distilled water and dilute to 1 liter. If necessary, standardize against 0.1 *N* $Na_2S_2O_3$, *Methods of Analysis, A.O.A.C.*, 1935, 551, 26(c).

(e) *Sodium thiosulfate.*—0.1 *N*. Standardize as directed in *Methods of Analysis, A.O.A.C.*, 1935, 542, 3(b).

(f) *Starch indicator.*—Mix 2 grams of finely pulverized potato starch with cold water to make a thin paste. Add 200 cc. of boiling water with constant stirring.

DETERMINATION

Weigh 0.18–0.20 gram of 2–4 dinitrophenol or its sodium compound into a tared beaker of about 100 cc. capacity, and dissolve the substance in 25 cc. of water and 5–10 cc. of 2% NaOH (do not heat). Transfer the solution to a 500 cc. glass-stoppered flask (an iodine flask is preferable), using water for washing. Dilute the solution with water to about 100 cc., add 25 cc. of the 0.1 *N* bromide-bromate solution and 10 cc. of HCl. Immediately stopper the flask and swirl vigorously for 1–3 minutes. Remove the stopper quickly and add 5 cc. of the KI solution, taking care to avoid loss of bromine; immediately stopper the flask and shake thoroughly for about 1 minute. Remove the stopper and rinse down the neck of the flask with the water. Titrate the solution with 0.1 *N* $Na_2S_2O_3$, using starch indicator near the end point.

1 cc. of 0.1 *N* bromide-bromate solution = 0.0092 gram of 2–4 dinitrophenol.

1 cc. of 0.1 *N* bromide-bromate solution = 0.0103 gram of sodium dinitrophenate.

1 cc. of 0.1 *N* bromide-bromate solution = 0.0112 gram of sodium dinitrophenate plus H_2O.

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[1] *Am. J. Pharm.*, 106, 462 (1934).

In Mixtures

Tablets.—Determine the average weight of at least 20 tablets. Grind in a mortar to a uniform powder.

Capsules.—Determine the average net contents of at least 20 capsules and mix the contents thoroughly.

Weigh into a tared beaker of about 50 cc. capacity a sample equivalent to about 0.18 gram of 2–4 dinitrophenol or its sodium compound. Macerate for a short time with 10 cc. of water and 10 cc. of NaOH solution. Transfer to a separator, wash beaker with water, and drain washings into separator. Acidify with HCl. Extract with 20 cc. of $CHCl_3$ and repeat until extraction is complete (usually 5 or 6 extractions are necessary), avoiding vigorous shaking, particularly during first 2 extractions. Test for complete extraction by shaking out the last $CHCl_3$ extraction with 5 cc. of the NaOH solution (a yellow color in the latter indicates incomplete extraction; 5 cc. containing 0.025 mg. is pale yellow).

Combine the $CHCl_3$ extracts in a separator and shake out with 10–15 cc. of the NaOH solution. Draw off the $CHCl_3$ layer into a third separator and repeat the extraction until no more yellow color is extracted. Note the total volume of NaOH solution used.

Transfer the alkaline solutions to a 500 cc. glass-stoppered flask, washing the separator each time with water. Add the exact quantity of HCl necessary, previously determined, to neutralize the NaOH solution used. Proceed as directed under "Determination," beginning with "Dilute the solution with water. . . . "

Table 3 gives results reported by the collaborators. Sample No. 1 was pure 2–4 dinitrophenol and Sample No. 2 contained 18.4 per cent of 2–4 dinitrophenol in a mixture of equal parts by weight of starch, lactose, and calcium carbonate.

TABLE 3.—*Results reported by collaborators*

COLLABORATOR	2-4 DINITROPHENOL FOUND	
	SAMPLE NO. 1	SAMPLE NO. 2
	per cent	*per cent*
Irwin S. Shupe	100.0	18 6
	100.1	18.5
E. H. Berry	99.8	18 3
	100 0	18.2
Albert I. Cohen	99 9	18 3
	100.3	18.4
Llewelyn Jones	100.1	18.4
	100.2	18.65
	100.0	—
M. Harris	99 8	18 2
	99.7	18.2
S. Reznek	100.2	18.4
	100.5	18 8
G. M. Johnson	99.6	—
	99.8	—

COMMENTS BY COLLABORATORS

Irwin S. Shupe.—In the extraction of the dinitrophenol with $CHCl_3$ I found it more convenient to use larger portions to avoid emulsions.

Albert I. Cohen.—It is suggested that the directions be made more explicit in regard to duration of maceration.

M. Harris.—No difficulty was encountered with the method when caution was

taken to avoid emulsification in the $CHCl_3$ extraction. It is suggested that a test for complete extraction with $CHCl_3$ by shaking the last $CHCl_3$ extraction with 3 cc. of NaOH solution, yellow color in the latter indicates incomplete extraction, be given.

S. Reznek.—**The method seems fairly satisfactory. Somewhat larger quantities of $CHCl_3$ than specified cut down emulsification. It might be well to give details as to how to determine the neutral point.**

The suggestions made by the collaborators have been included in the proposed method.

SUMMARY

(1) A critical investigation of the excess bromination method for 2–4 dinitrophenol was made, and the conditions, within reasonable limits for accurate quantitative result, were determined.

(2) The quantity of excess bromine plays only a subordinate role. The bromination period is more important—a longer time than given in the proposed method gives an error of about +4 per cent. The acid concentration is most important—a lower acid content gives an error of as much as 80 per cent.

(3) Accurate methods for the quantitative determination of 2–4 dinitrophenol or its salt, singly and in admixture, were developed.

(4) The methods were submitted for collaborative study, and results are included.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods presented by the Associate Referee for 2–4 dinitrophenol or its sodium compound, singly or in mixtures, such as tablets and capsules, be adopted as tentative, and later as official, without further experimental work.

(2) That the subject of 2–4 dinitrophenol, singly or in mixtures, be considered closed.

No report on theobromine in theobromine calcium tablets was given by the associate referee.

No report on chlorbutanol was given by the associate referee.

REPORT ON PHENOLPHTHALEIN AND ACETYLSALICYLIC ACID

By GEORGE M. JOHNSON (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

This is the first year that collaborative work has been done on this subject. A review of the literature showed the paper by Hitchens,[2] in

[1] For report of Subcommittee B and action of the Association, see *This Journal*. 20 57 (1937).
[2] *J. Am. Pharm. Assoc.*, 23, 1084 (1934).

which he discusses the determination of acetylsalicylic acid in mixtures with various organic compounds, one of which is phenolphthalein. In this method the powdered material is extracted by chloroform and the acetylsalicylic acid separated by sodium bicarbonate solution, from which, after acidification, the acetylsalicylic is extracted by an appropriate solvent.

The Associate Referee thought that this method could be adapted to the separation and determination of both phenolphthalein and acetylsalicylic acid without much change after making allowances for the slight solubility of phenolphthalein in chloroform and the small percentage of phenolphthalein in such mixtures. The following procedure was formulated:

About one gram of the material is mixed with a saturated solution of sodium bicarbonate, and the phenolphthalein is extracted with chloroform-ether solvent (2:1). The bicarbonate solution is acidified and the acetylsalicylic acid extracted with chloroform. The phenolphthalein is determined by weight and as the iodo compound and the acetylsalicylic acid by weight and double titration.

Since this method gave good preliminary results for the Associate Referee, a mixture containing phenolphthalein, acetylsalicylic acid, and starch was sent to the collaborators. Their reports on the mixture were disappointing. The results on the phenolphthalein by weighing were in fair agreement, both with the amount in the mixture and among the collaborators. By the iodo method, however, the results were much lower. The acetylsalicylic acid results were low by both methods and the collaborators did not check well among themselves.

Although it is apparent that the method as it stands is not entirely satisfactory, nevertheless it shows promise, and accordingly it is recommended[1] that it be given further study.

REPORT ON HOMATROPINE TABLETS

By EDWARD M. HOSHALL (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

The artificial tropeine, homatropine (mandelyl tropeine $C_6H_5CH(OH) \cdot COO \cdot C_8H_{14}N$), is a lower homologue of atropine in which the $-CH_2OH$ group is replaced by the hydroxyl group. It is prepared by the interaction of mandelic acid and tropeine in the presence of hydrogen chloride and water.

Ophthalmic tablets from 1/400 grain upwards, hypodermic tablets from 1/250 grain upwards, dispensary tablets of 1/2 grain or more, and lamella or discs of 1/100 grain (B.P. 1932) are among the types of homatropine

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 57 (1937).

tablets commercially available. A mixture of homatropine salts and cocaine hydrochloride is prepared in ophthalmic and dispensary tablets, with total content varying from 1/20 to 2 grains and percentage of homatropine from 10 to 50 per cent.

In order to determine the homatropine content of these tablets and other similar tablets in use, and to anticipate to some extent tablets that may be prepared in the future within this same class, the study of this problem was divided into three sections as follows:

I. The assay of homatropine in the commercially available alkaloid and its salts.

II. The assay of tablets of homatropine, prepared with such fillers, diluents, and lubricants as lactose, gelatin, stearic acid, glycerol, talc, and starch.

III. The assay of homatropine in the presence of cocaine.

EXPERIMENTAL

The assay of homatropine

I.—A sample of commercial U.S.P. homatropine hydrobromide was obtained, and the product was found to conform in all particulars to the requirements of U.S.P. XI (p. 185) tests for identity and purity.

A portion was admixed with 1 per cent by weight of atropine, and the U.S.P. XI tests for purity showed the presence of this alkaloid. Additional qualitative tests used to differentiate homatropine from atropine, hyoscyamine, and scopolamine were employed, and none was found more sensitive than those of the U.S.P. XI.

In order to follow the process of extraction of the alkaloid by means of a volatile solvent, it was ascertained that Wagner's reagent, *Methods of Analysis, A.O.A.C.*, 1935, p. 543, was sensitive 1:6000; Mayer's reagent, U.S.P. XI, p. 553, 1:3500; and the U.S.P. XI test was impracticable under the testing conditions. Wagner's reagent, therefore, was selected for making the necessary qualitative tests in order to ascertain the completeness of extraction.

I-a.—Analysis of the U.S.P. salt was made by extraction of the liberated base with a volatile solvent, with subsequent volatilization of the solvent, addition of excess acid, and titration of the excess acid. The A.O.A.C. tentative method for atropine in tablets, *Methods of Analysis*,

TABLE 1.

	TAKEN	FOUND	RECOVERY
	gram	*gram*	*per cent*
Homatropine hydrobromide	0.1500	0.1491	99.4
	0.1500	0.1484	98.9
	0.3000	0.2979	99.3
	0.3000	0.2970	99.0
Homatropine	0.1500	0.1481	98.7

A.O.A.C., 1935, p. 555, with a few changes, was used, namely, test for complete removal after fifth extraction with Wagner's reagent. Care should also be taken to see that the excess acid is added before the volume of the solvent falls lower than 5 cc., and this volatile solvent should be evaporated on a steam bath with the aid of a fan. Methyl red was selected as an indicator.

Determination of homatropine in tablets

II.—Practically all commercial tablets of this alkaloid or its salts are either dispensary, hypodermic, or ophthalmic types. An exception is the B. P. lamella or disc. In order, however, to anticipate future use of other diluents, fillers, lubricants, etc., various tablet mixtures were prepared and assayed by the method under I-a with results as follows:

TABLE 2.

HOMATROPINE HYDROBROMIDE TAKEN		FOUND	RECOVERY
gram	*gram*	*gram*	*per cent*
0.15	+0.4 lactose	0.1480	98.6
0.15	+0 8 lactose	0.1482	98.8
0.15	+0.8 talc	0.1482	98.8
0.15	+0.8 potato starch	0.1476	98.4
0.15	+1.5 U.S.P. gelatin	0.1466	97.7
0.15	+0.8 U.S.P. stearic acid	0.1488	99.2
0.15	+1.5 U.S.P. glycerol	0.1667	111.1

In the presence of glycerol and to some extent gelatin, the method above is not satisfactory. Accordingly a double extraction is necessary. The combined extracts of I-a are placed in a separator, made acid with 1 per cent sulfuric acid, and extracted five times with 1 per cent sulfuric acid, 15, 15, 10, 10, and 5 cc. being used. The aqueous extracts are combined, washed once with 2 cc. of chloroform and then made ammoniacal with 5 per cent ammonia. The solution is then extracted and titrated as in I-a. The following mixtures were prepared and assayed by this double extraction method:

TABLE 3

HOMATROPINE HYDROBROMIDE TAKEN		FOUND	RECOVERY
gram		*gram*	*per cent*
0.15		0 1465	97.7
0.30		0.2938	97.0
0.15	+1.0 grams glycerol	0.1476	98.4
0.15	+1.0 grams gelatin	0.1479	98.6

This latter double extraction method is satisfactory for the analysis of homatropine or its salts in tablets or lamella of normal composition.

III.—The method outlined above is not applicable to the determination of homatropine in the presence of cocaine.

SUMMARY

The A.O.A.C. tentative method for atropine in tablets, modified by a few minor changes, is applicable to the determination of homatropine in most types of commercially available tablets of homatropine or its salts. If gelatin and/or glycerol is present, as is the case in B.P. (1932) lamella, the same method is used except that the chloroform extract is extracted with acid, the acid solution is made ammoniacal, and extraction takes place as in the original method.

It is recommended that this study be continued.

REPORT ON CUBEB

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

A method for the evaluation of cubeb based upon results reported in *This Journal*, **19**, 411 (1936), was followed in the present collaborative work. The method submitted to the collaborators was published in *This Journal*, **19**, 98 (1936).

To determine the practicability of this method a coarsely ground sample of cubeb was submitted to O. C. Kenworthy and B. Lubell, New York Station, Food and Drug Administration. The results of assay are shown in the following table.

	Clevenger	*Kenworthy**	*Lubell*
Resins (%)	8.12	9.66	7.94
Volatile oil (cc. per 100 grams)	17.40	14.60	17.62
Specific gravity of the oil, (25°/25°)	0.919	0.914	0.913
Optical rotation of the oil†	−26.9	−23.5	−25.3
Refractive index of the oil (20°C.)	1.494	1.495	1.493
Acid No.	0.84	1.9	1.1
Ester No.	20.2	11.0	10.4

* Analyses made 10 months after material was ground and stored in a sealed glass jar.
† Angular degrees, 25°C., 100 mm. tube, white light.

Reasonable agreement in the yield of oleoresins (resin and volatile oil) of cubeb was obtained. The variation in these yields and the constants for the volatile oils are probably accounted for by the variation in the elapsed time between grinding the cubeb and the assay of materials.

It is recommended[1] that the method presented be adopted as tentative and that further work be done.

REPORT ON METHODS FOR TITRATION OF ALKALOIDS

By R. L. HERD (U. S. Food and Drug Administration, Buffalo, N. Y.), *Associate Referee*

A number of discrepancies have been encountered in the assay for the more common alkaloids, namely, strychnine, morphine, codeine, atropine, procaine, cocaine, and quinine. Different analysts, using the same method, have obtained varying results on the same product. Also different meth-

[1] For report of Subcommittee B and action of the Association, see *This Journal*, **20**, 57 (1937).

ods have given altogether inconsistent results. A review of the methods adopted by the A.O.A.C. for alkaloidal assay has revealed a number of inconsistencies, which contribute to erroneous results. In view of these difficulties, this Association appointed an Associate Referee, whose duty is to unify the methods for the assay of alkaloids.

No provision is made for the purification of the alkaloid before the final determination is made, except perhaps for morphine. In their work on atropine sulfate tablets, Watkins and Palkin, *This Journal*, **10**, 130 (1927), show that if magnesium and calcium compounds are present together with stearic acid the respective stearates are extracted from an alkaline solution and are titrated as additional base, and thus erroneous results are obtained. They have offered a method for eliminating stearates as well as excessive excipient, but it seems limited in its applicability. In the official methods for strychnine and emetine the analyst is directed to dissolve the free base in alcohol and titrate with standardized sulfuric acid to a faint red end point with methyl red as indicator. Recently boiled water is then added and the titration finished when the standard acid is added to a faint red end point. In the case of cocaine, pilocarpine, atropine, and procaine an excess of acid is added and the alkaloid is determined by back titration with standard alkali. In the case of codeine, the methods are offered as optional, *Methods of Analysis, A.O.A.C.*, 1935, 450, 452, 461–2, 463–4, 470, 472, 474. Methyl red is used for nearly all the alkaloids, but the end point of the reaction is not clearly defined. When the volume of the standard acid consumed is large, the variation due to an indefinite end point is not appreciable, but if one grain or less of the alkaloid is titrated the error may become pronounced. Because of variation in visual recognition of color change, different analysts may have entirely different views regarding the completion of the reaction.

The *p*H value of various alkaloidal salts has been determined by different operators with varying results. H. Wales[1] has determined many of these and compiled a table of *p*H values of neutral salts, suitable indicators, and their *p*H range, some of which are as follows:

Alkaloid	*p*H	*Indicator Range*	*Indicator*
Atropine	5.56	3.8–7.2	Methyl red, Propyl red, or Bromcresol blue
Cocaine	5.20	4.0–6.5	Methyl red
Codeine	4.86	3.6–6.3	Methyl red
Emetine	4.90	4.2–5.6	Methyl red
Morphine	4.86	4.0–5.0	Methyl red
Quinine	6.10	5.5–6.5	Bromcresol purple
Strychnine	4.81	3.8–6.0	Methyl red

Kolthoff[2] applied the conductometric method and the potentiometric method and obtained similar *p*H values. Kolthoff,[2] Wagener and McGill,[3]

[1] *Ind. Eng. Chem.*, **18**, 390 (1926).
[2] *Biochem. Z.*, **162**, 289 (1925).
[3] *J. Am. Pharm. Assoc.*, **14**, 288 (1925).

Rasmussen and co-workers,[1] and Pideaux and Winfield[2] applied potentiometric titrations to alkaloids. Wagener and McGill also compared potentiometric with indicator titrations. In these determinations the anhydrous free alkaloid was dissolved in an excess of standard acid, quinhydrone and methyl red indicator was added, the solution then diluted to 250 cc. with carbon dioxide free water, and back titrated with standard alkali. They found that the indicator changed color before the electrometric end point was reached. The results obtained by the color indicator were nearer the theoretical than were those obtained by the potentiometric titration. They give results on only one determination as a basis for this conclusion. They also show that the *p*H values of the neutral alkaloids vary noticeably with dilution, but the presence of salt, which is formed during titrations, has but slight affect on the *p*H value. Various other methods have been presented for the determination of alkaloids. Hatcher and Hatcher[3] used standard bromine solution and calculated the quantity of alkaloid from the amount of bromine consumed. Prescott and Gordon[4] offered an iodometric method, which was adopted by Allen as a rapid assay of hypodermic tablets of strychnine. Methods specifying bromine or iodine have restricted applicability due to the fact that they react with many substances other than alkaloids. Allen[5] gives a method based on titration with Mayer's reagent that seems worthy of consideration, especially for morphine. Gravimetric methods have been used to advantage in cases where the alkaloids can be isolated in a pure state, but the analyst cannot always be assured that the alkaloids being weighed are pure. Therefore the acidimetric titration seems of more value for a general assay than any of the other methods advocated.

The Associate Referee has been occupied with the purification of certain alkaloids that will be used subsequently for comparing and unifying the titration methods of analysis. The results obtained will be reported later.

RECOMMENDATIONS[6]

It is recommended—

(1) That collaborative study be made on purification of alkaloids by double extraction.

(2) That titration methods for alkaloids be studied, particularly for the purpose of defining the end point and selecting the proper indicator.

CORRECTION

In *Methods of Analysis, A.O.A.C.*, 1935, p. 225, "(1+1)" in line 4 of the paragraph beginning "Weigh duplicate samples," should read "(1+4)."

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[1] *Pharm. Z.*, 65, 729 (1924).
[2] *Analyst*, 55, 561 (1930).
[3] *J. Am. Pharm. Assoc.* 24, 262 (1935).
[4] Allen, Commercial Organic Analysis, 5th Ed., vii, 797.
[5] *Ibid.*, 29–31.
[6] For report of Subcommittee B and action of the Association, see *This Journal*, 20, 58 (1937)

CONTRIBUTED PAPERS

THE COLORIMETRIC DETERMINATION OF LACTIC ACID IN FRUITS AND FRUIT PRODUCTS

By FRED HILLIG (Food Division,* U. S. Food and Drug Administration, Washington, D. C.)

The lactic acid content of a fruit product may have significance to the regulatory chemist in detecting adulteration. Lactic acid in excess of that normal to a fruit product may have been directly added as such, or indirectly through the use of pectin acidulated with lactic acid.

Accordingly it seemed desirable to test the applicability of the writer's colorimetric method for the determination of lactic acid in milk and milk products[1] to fruit and fruit products. This paper is a report on this study.

It was found that the dairy products method could be readily applied to the determination of lactic acid in fruit and fruit products. The principal steps in this procedure are (1) extraction of the lactic acid, (2) purification of the extracted acid with carbon, (3) development of the characteristic yellow color, and (4) final estimation of lactic acid. The original paper gives detailed information regarding reagents, the extractor used, reasons for the carbon treatment, the mechanics of the development of the characteristic yellow color with ferric chloride, and the final estimation of lactic acid photometrically or in Nessler tubes.

A filter centering around 460 mμ instead of one centering around 450 mμ was used in making the photometric comparisons because it was thought it might not be possible with all photometers to place a piece of Noviol yellow glass in the light beam traversing the wedge of the instrument. With a filter of 460 mμ the hue difference is greater than it is with a filter of 450 mμ, but the analyst should have no difficulty in making good readings. The method follows.

LACTIC ACID IN FRUITS AND FRUIT PRODUCTS

Weigh 50 grams of a jam or jelly into a 400 cc. beaker, add about 150 cc. of water, and boil gently for 15 minutes. Cool, transfer to a 250 cc. volumetric flask with water, make to mark, shake, and filter through cotton. Transfer 50 cc. of the filtrate to the continuous extractor, add 1 cc. of H_2SO_4 (1+1), and proceed with the determination as directed in the original paper, except to extract 3 hours instead of 2 hours. In the case of fruit juices, transfer 10 cc. of the material directly to the extractor, add 40 cc. of water and 1 cc. of H_2SO_4 (1+1) and proceed as directed above.

Compute the quantity of material in the final aliquot as follows:

$$\frac{50}{250}\times\frac{50}{110}\times\frac{100}{55}\times A=0.1653A,$$

in which A equals cc. of aliquot taken in the final analysis.

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* W. B. White, Chief.
[1] *This Journal*, **20**, 130 (1937).

The same formula (0.1653A) is also used when 10 cc. of a fruit juice is transferred to the extractor.

In order to test the accuracy of the method, varying quantities of lithium lactate were added to a commercial grape juice and to an authentic grape jelly. Recoveries are shown in Table 1.

TABLE 1.—*Recovery of lactic acid by the proposed method*

LACTIC ACID ADDED	LACTIC ACID DETERMINED	LACTIC ACID RECOVERED	
mg.	*mg.*	*mg.*	*per cent*
Added to grape juice (lactic acid in 10 cc. juice, 0.84 mg.)			
1	1.74	0.90	90.0
	1.77	0.93	93.0
5	5.85	5.01	100.2
	5.79	4.95	99.0
10	11.04	10.20	102.0
	10.83	9.99	99.9
25	25.52	24.68	98.7
	25.36	24.52	98.1
40	40.26	39.42	98.6
	40.00	39.16	97.9
50	49.43	48.59	97.2
	49.54	48.70	97.4
60	59.89	59.05	98.4
	59.77	58.93	98.2
Added to grape jelly (lactic acid in 10 g jelly, 0.78 mg.)			
2	2.81	2.03	101.5
	2.81	2.03	101.5
5	5.76	4.98	99.6
	5.76	4.98	99.6
10	10.56	9.78	97.8
	10.80	10.02	100.2
25	25.51	24.73	98.9
	25.51	24.73	98.9
30	30.41	29.63	98.8
	30.26	29.48	98.3
40	39.73	38.95	97.4
	39.93	39.15	97.9
50	50.82	50.04	100.1
	51.12	50.34	100.7

The closely agreeing duplicates obtained (Table 1) show that the method delivers practically all the lactic acid added. The fact that the "blank" is an important fraction of the added lactic acid at the 1 mg. level perhaps furnishes less justification for calculating "per cent recovery" than in those cases where 5 mg. or more lactic acid was added.

The lactic acid content of a large number of authentic fruits was determined by the proposed method. The results are given in Table 2.

TABLE 2.—*Lactic acid in authentic fruits determined by the proposed method*

FRUIT	LACTIC ACID	FRUIT	LACTIC ACID
	mg./100 g.		*mg./100 g.*
Apple-Stayman	9.1 8.8	Orange-Seedless California	3.5* 3.2*
Apple-Winesap	5.6 5.3	Peach—Elberta	13.2 13.2
Apricot-Royal	8.7 9.4	Peach—Tuscan	10.8 11.5
Blackberry—Mammoth	20.2 19.3	Pineapple—Hawaiian	10.5 11.3
Blackberry—Macatawa	19.8 17.3	Pineapple—Hawaiian	14.8 14.8
Blackberry—Himalya	15.0 14.3	Plum—Burbank	17.2 16.1
Cherry—Red Sour	8.4 8.6	Plum—Damson	32.1 33.7
Crab Apple—Hyslop	18.3	Plum—Satsuma	13.3 13.3
Currant—Red	6.1 6.1	Plum—Sugar	24.1 23.0
Fig—Kadota	20.2 18.6	Quince—Apple Variety	4.4 4.9
Grape—Concord	13.4 13.4	Raspberry—Black	10.3 10.0
Grape Fruit	9.7* 9.5*	Raspberry—Red	12 8 12.8
Lemon	7.9* 7.9*	Strawberry—Banner	15.2 15.0
Loganberry	14.1 14.5	Tomato	5.7†
Orange—Valencia Florida	6.2* 6.2*	Youngberry	18.1 17.2

* Mg. per 100 cc. of juice.
† Mg. per 100 cc. of juice. Average of 13 samples.

It will be seen (Table 2) that the method yielded small quantities of lactic acid in all the authentic fruits analyzed.

Lactic acid was also determined in a large number of commercial jams, jellies, and preserves by the proposed method. The results are given in Table 3.

TABLE 3.—*Lactic acid in commercial fruit products determined by the proposed method*

PRODUCT	LACTIC ACID	PRODUCT	LACTIC ACID
	mg./100 g.		*mg./100 g.*
Apricot Preserves	58.1 57.5	Pectin Liquid, acidified	3391.0 3351.0
Blackberry Preserves	129.3 125.9	Pectin Liquid	2705.0 2700.0
Blackberry Jelly	284.4 283.5	Pectin Dry	45.4 44.2
Blackberry Jam	94.1 91.5	Pineapple Preserves	15.4 15.7
Cherry Preserves	110.4 115.4	Plum Preserves	65.1 65.8
Cherry Preserves	157.5 148.3	Plum Jelly	278.7 284.7
Currant Jelly	198.2 198.2	Quince Jelly	241.4 241.1
Currant Jelly	259.6 261.7	Raspberry Preserves	12.8 13.2
Currant Jelly	8.0 8.8	Raspberry Preserves	255.4 251.7
Grape Juice	5.3* 5.5*	Raspberry Preserves	19.4 19.4
Grape Juice	6.9* 7.0*	Raspberry Jelly	273.8 276.2
Grape Juice	8.6* 8.2*	Raspberry Jam	77.8 77.0
Grape Jelly	7.9 7.6	Raspberry Jam	17.5 16.7
Grape Jelly	298.3 292.0	Raspberry Jam	6.1 6.1
Grape Preserves	120.7 119.1	Strawberry Preserves	275.3 271.7
Orange Marmalade	30.0 30.3	Strawberry Preserves	70.6 70.0
Peach Preserves	134.7 134.0	Strawberry Preserves	32.2 32.2
Peach Preserves	74.1	Strawberry Jelly	238.4 243.9
Pectin Liquid, not acidified	37.0 36.6		

* Mg. per 100 cc. of juice.

TABLE 4.—*Lactic acid in wines determined by the proposed method*

KIND AND ORIGIN	VINTAGE	LACTIC ACID
		mg./100 cc.
Angelica—California	?	186.7
Burgundy—California	1927	307.7 306.8
Cabernet—California	1925	338.3 344.9
Catawba-Sweet—New York	1928	341.0 346.1
Chablis—California	1925	328.9 334.9
Chateau-Yquem—California	1931	276.9 277.8
Claret—California	1933	279.3 278.1
Muscatel—California	?	240.5 240.5
Port—New York	1933	371.3 369.3
Port—New York	1933	208.8 202.1
Riesling—New York	1933	106.8 109.4
Johannisburg—Riesling—California	1933	189.1 193.6
Haut-Sauterne—New York	?	269.9 276.9
Sauterne—California	?	311.3 306.8
Sherry—California	?	305.6 306.2
Tokay—New York	1915	288.3 298.9
Zinfandel—California	1933	485.5 484.2

No attempt was made to obtain a true cross section of commercial jams and jellies. A number of the samples were deficient in the fruit content exacted by the Federal Standards for Jam and Jelly. These samples invariably showed a high lactic acid content. In some instances the lactic acid was doubtless added as commercial pectin acidulated with lactic acid, in others it was probably added as an acidulant.

WINES

Lactic acid is a constituent of normal wines, being derived from malic acid during fermentation. Owing to the tendency of some wines, especially the sweet wines, to foam in the extractor when attempts are made to extract them directly, it is necessary to give the material a preparatory treatment different from that used for jams and jellies. The material in the wine causing this troublesome foaming can be removed by precipitation with alcohol. The procedure follows:

LACTIC ACID IN WINES

Transfer 50 cc. of wine to a 250 cc. volumetric flask, add about 100 cc. of alcohol, and shake vigorously. Make to mark with alcohol and filter through a folded paper. Transfer 200 cc. of the filtrate to a 400 cc. beaker and evaporate on a gauze to about 25 cc. Add 50 cc. of water and again evaporate to about 25 cc. Transfer to a 200 cc. volumetric flask with water, make to mark, shake and pipet 50 cc. into the extractor. Add 1 cc. of H_2SO_4 (1+1) and proceed as directed for fruit and fruit products.

Lactic acid was determined in a number of wines by the proposed method. The results are given in Table 4.

SUMMARY

A method is proposed for the colorimetric determination of lactic acid in fruit and fruit products. The various steps in the procedure are not difficult, and the determination can be completed easily in a working day.

A STUDY OF MANUFACTURING CONTROL METHODS FOR ESTIMATION OF SULFUR DIOXIDE IN ALCOHOLIC BEVERAGES*

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A number of standard methods for the determination of sulfur dioxide in foods and beverages are available for use by the analytical chemist, but the choice of method depends upon a number of factors. In general, for manufacturing or control purposes, a method must permit speed of operation without serious sacrifice of accuracy.

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* Presented at the meeting of the American Chemical Society held at Omaha, Neb., April, 1937.

Nichols and Reed[1] and Monier-Williams[2] have made careful reviews of this subject, therefore none need be included here. Factors such as the nature of the food product, the level of sulfur dioxide contained therein, the type of acid used for evolution, the absorbent and oxidizing medium, and even the mechanical arrangement or construction of laboratory apparatus apparently affect the results.

In the course of the work presented here it was necessary to find a method which, under specific laboratory conditions, would give accurate and reproducible estimations of sulfur dioxide in alcoholic and carbonated beverages.

STANDARD METHODS

The four standard methods studied were the Monier-Williams;[3] the direct distillation;[4] the A.O.A.C. steam distillation;[5] and the Ripper.[6] The principles and procedure of these methods and the modifications used are briefly described.

Monier-Williams Method.—The sample is distilled for a given length of time under a reflux condenser and the sulfur dioxide is swept into cold hydrogen peroxide by a current of carbon dioxide. Hydrochloric acid is used to acidify the product, the reflux condenser preventing the acid from distilling over. The cold hydrogen peroxide oxidizes the sulfurous acid to sulfuric acid quantitatively, thus permitting the final determination of the sulfur dioxide in the product either by the gravimetric barium sulfate precipitation or by the titration of the sulfuric acid with a standard solution of sodium hydroxide.

Direct Distillation Method.—The distillate from the acidified sample is collected in an oxidizing agent. Hydrochloric acid was used as the liberating medium, because it was shown by Nichols and Reed[7] that this acid is more efficient than the phosphoric acid specified in the method. Either bromine or iodine may be used as the oxidizing medium. The oxidized sulfur dioxide is then determined gravimetrically when bromine is used, or by the back titration of the excess oxidizing agent by standard sodium thiosulfate if iodine is employed. One gram of sodium bicarbonate is added to the distilling flask before each distillation to sweep out any oxygen, which might react with the liberated sulfur dioxide.

A.O.A.C. Steam Distillation Method.—Essentially the same as the direct distillation method except that steam is introduced into the distilling flask and is used as a heating medium and also to assist in carrying over the released sulfur dioxide into the bromine, which is used as the oxidizing medium. Phosphoric acid is employed as the evolving medium, and the

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[1] *Ind. Eng. Chem Anal. Ed.*, **4**, 79 (1932).
[2] Reports on Public Health and Medical Subjects No. 43, Ministry of Health (1927).
[3] *Methods of Analysis, A O A.C*, 1935, 440.
[4] *Ibid*, 1930; McCharles and Pitman, *Ind Eng Chem Anal. Ed*, **8**, 55 (1936).
[5] *Methods of Analysis, A.O.A.C.*, 1935, 152.
[6] Bigelow, U. S. Dept. Agr. Bur. Chem Bull. **65** (1902).
[7] *Loc. cit.*

gravimetric barium sulfate precipitation method is used for the final determination of the resulting sulfuric acid.

Ripper Method.—Recommended only for wines and beer. It specifies treatment of the product with dilute caustic solution, which breaks down the aldehyde-sulfite compounds; the addition of dilute sulfuric acid to liberate the sulfurous acid; and the titration of the liberated sulfurous acid with standard iodine solution.

EXPERIMENTAL PROCEDURE

In each instance, the methods outlined were applied to sulfur-free water, ales, beers, and several types of wine after the addition of known amounts of standard sulfurous acid solution. The sulfurous acid used was reagent quality diluted to the desired concentration, which was checked by the method described by Scott[1] immediately before it was added to the sample. Blanks were run and subtracted from the results in each instance.

Monier-Williams Method

The Monier-Williams method has the following advantages over other methods; (1) By refluxing, the frothing sometimes experienced with certain products is reduced; (2) organic acids are retained in the distilling flask; (3) errors due to other volatile sulfur compounds are reduced; and (4) sulfur dioxide is liberated more completely. The method is time consuming and requires the use of carbon dioxide. However, because accuracy was considered to be more desirable than speed, and because of its generally accepted reliability the Monier-Williams method was selected as a basis for this comparison of methods. First, recovery tests were carefully made on aqueous solutions by the detailed Monier-Williams method. These data are presented in Table 1.

TABLE 1.—*Sulfur dioxide recoveries from aqueous solutions by the Monier-Williams method (distillation time, 60 minutes)*

TRIAL NO.	ADDED SO_2	SO_2 RECOVERED			
		GRAV.	VOL.	GRAV.	VOL.
	mg.	*mg.*	*mg.*	*per cent*	*per cent*
1	3.57	3.66	3.84	103	107
2	3.57	3.41	3.68	96	103
3	3.87	—	3.84	—	99
4	3.77	—	4.00	—	106
5	2.10	2.10	2.30	100	109

Preliminary tests were also conducted on ale to determine the size of sample to use as well as the most desirable refluxing period. It was found (Table 2) that 300 ml. samples worked satisfactorily, and that a distilla-

[1] Standard Methods of Chemical Analysis, 4th Ed., 512.

tion time of 90 minutes was more satisfactory than one of 60 minutes. Unless given otherwise later the sample volume will be understood to be 300 ml.

TABLE 2.—*Sulfur dioxide recoveries from ale by the Monier-Williams method*

TRIAL NO.	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED			
	mg.	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
		Distillation time, 60 minutes				
1	5.44	2.50	1.84	1.71	74	68
2	5.44	2.88	2.91	2.87	101	100
3	1.83	2.47	2.23	2.33	90	94
4	9.20	2.47	1.30	—	53	—
5	8.01	2.72	2.11	—	77	—
		Distillation time, 90 minutes				
1	7.85	3.40	3.08	3.45	91	101
2	7.36	3.32	3 50	3.37	105	101
3	8.50	2.36	2.68	2.40	113	102
4	8.30	2.78	2 90	2 90	104	104
5	7.52	3.35	3.06	3 06	91	91

The method was further tried on beer. The recoveries (Table 3) indicate that this procedure is quite satisfactory for beer, as well as for ale.

TABLE 3.—*Sulfur dioxide recoveries from beer by the Monier-Williams method (distillation time, 90 minutes)*

TRIAL NO.	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED			
			GRAV.	VOL.	GRAV.	VOL.
	mg.	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
1	5.27	4.49	4.42	4.54	98	101
2	4.44	3.95	3.66	—	93	—
3	5.44	3.19	3.24	3.20	101	100

The Monier-Williams method was next applied to Madeira wine. Table 4 shows that 300 ml. samples also give satisfactory recoveries with a 90-minute refluxing period and gravimetric determinations of sulfate. The titration method for determining the oxidized sulfur dioxide could be applied to one sample only probably because of acids carried over into the absorption medium during refluxing, a condition believed to be associated with the use of too large a sample. Upon reducing the quantity of wine used for the sample to 100 ml. and diluting with sulfur-free water to 300 ml., the difficulty of using a titration method was not experienced and satisfactory recoveries were obtained, as is also indicated in Table 4.

TABLE 4.—*Sulfur dioxide recoveries from Madeira wine by the Monier-Williams method (distillation time, 90 minutes)*

TRIAL NO.	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED GRAV.	VOL.	GRAV.	VOL.
	mg.	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
			Sample volume, 300 ml.			
1	1.43	3.53	3.43	3.36	97	95
2	18.3	3.52	3.60	—	102	—
3	18.4	3.57	3.20	—	90	—
4	17.8	3.57	4.00	—	112	—
			Sample volume, 100 ml. wine+200 ml. H_2O			
1	0.77	3.88	4.39	3.84	113	99
2	0.69	3.59	3.44	4.00	96	111
3	0.74	3.76	2.91*	3.52	77*	94

* Portion of sample lost.

To determine further the adaptability of the method to wines it was also applied to Sauterne (Table 5). Here also samples of 100 ml. gave satisfactory recoveries. Two distillation periods were used, namely, 60 minutes and 90 minutes. Although on this particular wine it was apparent that the shorter period was sufficient, experience gained on reflux time with beer shows that a 90 minute distillation period should be used on all products of this type unless recoveries that would indicate that the refluxing period might be reduced below this figure are obtained on each class of wine tested.

TABLE 5.—*Sulfur dioxide recoveries from Sauterne wine by the Monier-Williams method**

TRIAL NO.	DISTILLATION TIME	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED GRAV.	VOL.	GRAV.	VOL.
	minutes	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
1	60	19.10	3.52	3.12	3.84	89	109
2	60	18.60	3.36	3.49	3.52	104	105
3	90	24.4	3.81	3.50	3.40	92	89
4	90	23.6	3.59	3.40	3.70	95	103

* Sample Volume, 100 ml. wine+200 ml. H_2O.

Direct Distillation

The direct distillation method, with bromine as the absorption medium, was tried on aqueous solutions, beer, and wine. Satisfactory recoveries were obtained on aqueous solutions of sulfurous acid. When standard iodine solution was used as the absorption medium with subsequent titration of the excess iodine with standard thiosulfate solution this method appeared to be more satisfactory. Nevertheless, the erratic results obtained on all recoveries (Table 6) led to abandonment of this method.

It will also be noted that in the case of beer a maximum recovery figure of 440 per cent was recorded, with a minimum value of 45 per cent. These data indicate that in some instances the recovery is decidedly too high, and they lead to the conclusion that under some conditions substances other than sulfur dioxide were distilled into the absorption medium to react with the iodine.

TABLE 6.—*Sulfur dioxide recoveries by direct distillation method**

PRODUCT	SAMPLE VOLUME	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED	
	ml.	*mg.*	*mg.*	*mg.*	*per cent*
		Absorbent, bromine*			
Water	300	0.00	3.09	3.18	103
Water	300	0.00	3.14	3.27	104
Water	300	0.00	3.14	3.29	105
Water	300	0.00	3.14	3.16	100
Beer	300	3.60	3.65	2.71	74
Beer	300	3.21	3.65	4.72	129
Sauterne	50	9.20	3.31	3.30	100
Sauterne	50	8.81	3.33	2.89	87
Sauterne	100	20.40	3.53	3.80	108
Sauterne	100	21.60	3.45	2.80	81
		Absorbent, iodine†			
Water	300	0.0	3.15	3.30	105
Water	300	0.0	3.15	3.17	101
Water	300	0.0	3.15	3.30	105
Water	300	0.0	3.15	2.78	88
Ale	300	9.11	3 22	3.24	101
Ale	300	9.37	3.22	3.10	96
Beer	300	4.69	3.22	4.16	129
Beer	300	5.74	3.22	3.10	96
Beer	300	5.48	3.63	15.92	440
Beer	300	7.28	3.63	1.63	45
Sauterne	100	33.40	3.45	3.50	101
Sauterne	100	33.60	2.98	3.50	117
Madeira	100	5.48	3.27	2.92	89
Madeira	100	5.01	3.27	3.04	93
Sherry	100	5.08	3.27	3.24	99
Sherry	100	5.21	3 27	2.85	87

* Liberating acid, HCl; method, gravimetric.
† Liberating acid, HCl; method, volumetric

A.O.A.C. Steam Distillation Method

The A.O.A.C. steam distillation method is recommended for beer only, nevertheless it was tried on wines. At first, when excessive foaming was encountered, hydrocarbon oils were added, but they failed to prevent foaming and allowed some of the beer to be carried over into the absorption flask. Chemically pure olive oil worked satisfactorily for this purpose, but poor recoveries were obtained (Table 7). The oil may have prevented

the complete evolution of the sulfur dioxide because of mechanical interference or absorption of sulfur dioxide by some chemical reaction.

In the absence of oil, high recoveries were obtained on both wine and beer, which fact would indicate that volatile sulfur compounds other than sulfur dioxide were carried over into the absorption flask.

TABLE 7.—*Sulfur dioxide recoveries by the A.O.A.C. steam distillation method*

PRODUCT	SAMPLE VOLUME	INITIAL SO_2	ADDED SO_2	SO_2 RECOVERED	
	ml.	*mg.*	*mg.*	*mg.*	*per cent*
Beer	300	2.37	3.56	2.05*	58*
Beer	300	1.92	3.65	1.62*	44*
Beer	300	1.48	3.65	1.68*	46*
Beer	300	1.37	3.59	1.15*	32*
Beer	300	1.73	3.45	1.56*	45*
Beer	300	5.03	3.55	3.95	111
Beer	300	3.24	3.53	4.33	123
Beer	300	3.02	3.53	4.33	123
Sherry	100	5.65	3.90	3.33	85
Sherry	100	4.61	3.57	4.42	124
Sauterne	100	19.4	3.50	8.40	240
Sauterne	100	20.1	3.57	4.70	132

* Oil added.

TABLE 8.—*Comparison of Ripper and Monier-Williams methods for sulfur dioxide in wines, beer, and ale*

PRODUCT	RIPPER METHOD	M-W METHOD (GRAV.)	PRODUCT	RIPPER METHOD	M-W METHOD (GRAV.)
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*
Madeira	18	9	Tokay	16	2
Madeira	18	7	Tokay	18	1
Madeira	87	61	Muscatel	17	5
Madeira	86	59	Muscatel	18	4
Sherry	58	57	Malaga	31	8
Sherry	58	48	Malaga	34	5
Sauterne	166	191	Angelica	33	14
Sauterne	164	179	Angelica	34	11
Port	20		Beer	73	18
Port	21	6	Beer	73	17
Burgundy	48	42	Ale	65	29
Burgundy	49	41	Ale	69	28

Ripper Method

Many analysts still prefer to use the Ripper method for wines, although various reviews of the method indicate that it is unreliable. Leach[1] states that the method is suitable for white wines and beers, consequently it was included in this study. Results obtained by this procedure on various

[1] Food Inspection and Analysis, 4th Ed., 898.

types of wine (both white and colored), beer, and ale were compared with results obtained on the same products concurrently by the Monier-Williams method.

The results (Table 8) show that the method is totally unsuitable for beer and ale, and they are much higher than those obtained by the Monier-Williams method. With some types of wine the method might be sufficiently accurate, but it cannot be universally applied to all types.

CONCLUSIONS

Four standard methods for the determination of sulfur dioxide in food products were investigated for accuracy and practicability when applied to alcoholic and carbonated beverages. The results indicate that under the condition of the experiments the Monier-Williams method is the only one that is universally applicable to these products.

The authors wish to express their appreciation to R. W. Pilcher for his valuable suggestions and criticisms.

A STATISTICAL STUDY OF THE SAMPLING AND ANALYTICAL ERRORS ENCOUNTERED IN ANALYZING APPLES FOR LEAD SPRAY RESIDUES

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For several years the Division of Insecticide Investigations has cooperated with the Division of Fruit Insect Investigations of the Bureau of Entomology and Plant Quarantine and the Division of Fruit and Vegetable Crops and Diseases of the Bureau of Plant Industry in carrying on apple-washing experiments both in the East and in the Pacific Northwest. These experiments are made to get some idea of the effect upon ease of washing of such factors as type and number of cover sprays used; variety of apple; nature, concentration, and temperature of washing agent; time of exposure; type of washing machine, etc. It is obvious that the answers to all these questions depend primarily upon the use of a representative sample and a sufficiently accurate method of analysis.

Numerous attempts have been made to determine what constitutes a representative sample, all of them based on analyses made for arsenic by the Gutzeit method. This method is not highly accurate, and some of the investigators have not clearly disentangled its errors from the total error and thus have arrived at erroneous estimates of the difficulty of sampling. Youden[1] has given a clear summary of this situation. He reaches the general conclusion that the standard error of a single Gutzeit analysis (pre-

* The authors wish to acknowledge the assistance of C. R. Gross, C. W. Murray, and G A Holaday in the analytical work connected with the investigation.

[1] *Contrib. Boyce Thompson Inst.*, **3**, 363 (1931).

sumably one digestion, and one strip from one aliquot) is ±15 per cent, and indicates how to allow for this in studying variability of fruit.

With the development of the electrolytic dithizone method for the determination of small quantities of lead,[1] a new means of investigating the sampling error became available, and this paper gives the results of the study of four lots of apples.

The method just mentioned is delicate enough to permit the determination of the lead content of individual apples, even after washing. These studies were made on 100 single apples from each of three lots, and on 100 two-apple samples from the other lot. That the dithizone method is not only delicate but also precise was proved by testing it with unsprayed apples to which lead had been added.

Fifty unsprayed apples were peeled, and the peelings were put separately into 250 cc. beakers. To 25 of the beakers was added such an aliquot of a solution of lead nitrate as corresponded to about 0.250 grain per pound, and to each of 25 others a smaller aliquot containing the equivalent of about 0.026 grain per pound. All the material was mushed completely by the regular method with nitric acid, and then analyzed by the dithizone-electrolytic method. The method of analysis differed for the two quantities of lead, the lesser amount being determined with the aid of a smaller electrode and a more dilute thiosulfate solution. This modification is not followed when groups of washed apples are analyzed. The 25 that received the lesser amount of lead showed a standard deviation of 0.0009 grain per pound and the other lot showed a standard deviation of 0.003 grain per pound. These standard deviations are so low as to be negligible in comparison with the sampling errors encountered in the remaining phase of the work, and hence will not be considered in the statistical work.

STATISTICAL WORK

Following the harvest season of 1934, analyses were made on single unwashed Stayman Winesap apples selected at random from all parts of a single tree in an experimentally sprayed lot (A) at Kearneysville, W. Va. Another lot (B), of the Jonathan variety, experimentally sprayed and washed at Yakima, Wash., was also investigated, except that the apples, which again were picked at random from all parts of a single tree, were combined in pairs in order to facilitate analyses. In 1935 this work was repeated with some modifications. The apples were picked in four groups, about 120 being taken at random from the top half and 120 from the bottom half of each of two trees. One-half of the apples in each group were left unwashed, and 25 of these, selected at random, were analyzed separately. The other half in each group were washed in 1.5 per cent hydrochloric acid plus 0.5 per cent wetting agent in a Bean flotation washer

[1] *Methods of Analysis, A.O.A.C.*, 1935, 377–384.

for ¾ minute at about 70°F., and 25 apples were selected at random for individual analysis. An analysis of the variance of the results in this study showed no significant difference between trees or parts of trees, and therefore the results were grouped merely as representing washed and unwashed apples. However, large variances between trees have been encountered.[1] The results of all the work are summarized in Table 1 and shown graphically in Fig. 1. The curves in the chart show that this grouping was justified.

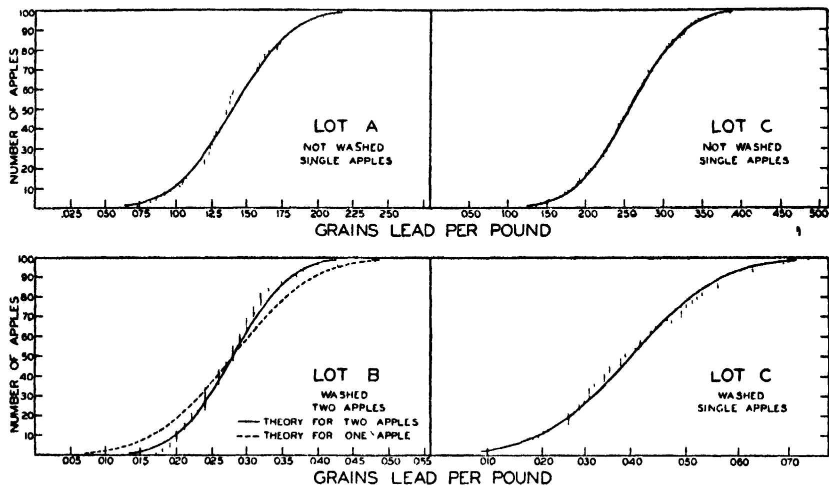

FIG. 1.—CUMULATIVE FREQUENCY CURVES, SHOWING DISTRIBUTION OF LEAD ON APPLES

The results of all the individual analyses are plotted (Fig. 1) in a manner to give cumulative frequency curves, and for each of the four groups the theoretical curve having the same mean and the same standard deviation as those of the group is shown. In the case of lot B, the additional broken curve shows theoretically the condition that would have been found if the apples had been analyzed singly rather than in pairs. Four different scales for residue values were used, so proportioned that the four means all fell equidistant from their respective Y axes. This makes the geometric slope of each curve at its mid-point inversely proportional to the coefficient of variation, thus permitting a visual estimate of that coefficient.

It is interesting to note (Table 1) that the coefficients of variation are practically the same, 22 and 24 per cent, for both lots of unwashed apples, and also approximately the same, 32 and 37 per cent, for both lots of washed apples, even though the means in each pair are appreciably differ-

[1] Haller, Cassil, and Gould, *J. Econ. Ent.*, 30, 174 (1937).

TABLE 1.—*Distribution of lead on apples sprayed with lead arsenate*

LOT	VARIETY	CONDITION	MIN.	LEAD CONTENT MAX.	AV.	STANDARD DEVIATION	COEFFICIENT OF VARIATION
				grain per pound		*grain per pound*	*per cent*
A	Stayman	Unwashed	0.073	0.214	0.140	0.033	24
B	Jonathan	Washed	0.017	0.050	0.028	0.0064	23
					0.028*	0.0090*	32*
C	Stayman	Unwashed	0.135	0.401	0.256	0.056	22
C	Stayman	Washed	0.014	0.074	0.039	0.0143	37

* Theoretically, these figures would have been obtained had the analyses been made on single apples.

ent. When it is remembered that the two sets of results for 1935 are on apples from the same lot, it appears possible that the washing operation has added a random error and thus increased the relative dispersion of the results.

If these results are to be used for estimating the size of sample necessary to insure within specified odds a desired accuracy, certain assumptions must be made regarding the dependence of the standard deviation on the quantity of spray residue present. The results quoted here suggest that the standard deviation should be considered to be proportional to the mean for both unwashed and washed apples, and that the proportionality factor, or coefficient of variation, is 30 per cent, on the average, for the unwashed and washed samples. Figure 2 was constructed from points calculated on this basis, as follows:

Let the two lots of apples to be compared have residues of M_1 and M_2, with a common mean of M. Assume that the coefficient of variation Cv is constant and equal to 30 per cent. Let $100\,\frac{M_2-M_1}{M}$ be represented as D per cent. Then if t represents the measure of significance, as used by R. A. Fisher, $N=\frac{2Cv^2t^2}{D^2}+\frac{Cv^2t^2}{20000}$. The second term is normally negligible, and was not considered in calculating the values in Fig. 2. Also only the significance levels 0.1, 0.05, and 0.01 were used. A logarithmic scale was used for the ordinates of this graph so that the curves might be extended to cover the upper range. This graph shows that if it is to be certified with a probability of 19:1 that two lots of unwashed apples are really different when their average loads differ by 10 per cent of their common mean, or that two washing treatments have given really different results (odds 19:1) when those results differ by 10 per cent of their common mean, each of the results must be obtained by the analysis of about 70 apples. It is believed that these estimates are the minimum. All the results reported here came from restricted sources, namely, one or two trees of a

plot sprayed carefully by a skilled operator. It seems quite certain that greater variability would be encountered in samples taken from commercially sprayed orchards. Because of inadequate sampling, it is believed that many of the results in the published literature do not wholly justify the conclusions drawn as to the relative effectiveness either of various spray schedules in building up spray deposits or of various washing procedures in removing it. It is hoped that this paper will lead to a better understanding of the sampling problem.

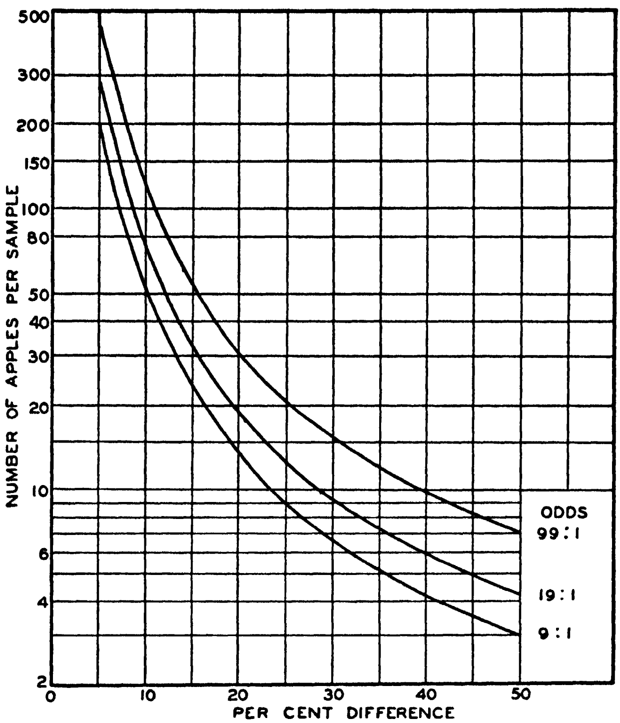

FIG. 2.—SIZE OF SAMPLE OF APPLES AS RELATED TO ACCURACY DESIRED IN DETERMINING LEAD RESIDUES

The remarks made concerning the small variability contributed by the analytical procedures may lead to the conclusion that unless extremely large samples are taken only one analysis is necessary. The sample should not be subsampled in the laboratory, but the peels from all the apples should be mushed together with acid, made to a known volume, and an aliquot taken for analysis. At present, however, it is the custom in this laboratory to use duplicate 30-apple samples to guard against the chance of loss of a determination, and also to give a measure of the sampling variability of the particular lots being analyzed.

SUMMARY

The distribution of lead among apples from trees sprayed with lead arsenate has been investigated by means of the dithizone electrolytic method, the error of which has been shown to be negligible in proportion to the apple sampling error. Some indication is given that the dispersion of the individual results is proportional to the size of the residue, and that it is increased when the apples are washed. A graph is given showing the size of samples necessary to show a difference between any two lots of apples.

DETERMINATION OF LEAD IN MAPLE PRODUCTS

By J. L. Perlman (State Food Laboratory, Department of Agriculture and Markets, Albany, N. Y.)

With the recognition of lead contamination in maple products it became necessary for the analyst to secure for its determination a method that would be accurate and adaptable to routine work, and at the same time rapid enough for making prompt analyses during the short harvest season.

The three principal sources of lead contamination in the production of maple sirup are lead-painted sap-gathering buckets, and hauling and sap storage tanks; leaded-tin or terne-plated equipment; and lead-soldered joints, particularly in tin-plated evaporators* where the sap is concentrated to one-fortieth or one-fiftieth of its original volume by open boiling. The third source mentioned is believed to be most likely to produce consistent lead contamination, owing to the prolonged heating necessary in intimate contact with the bare metal. In galvanized evaporators the zinc coating may prevent the solution of solder and perhaps even replace lead already in solution by electrochemical deposition.[1]

Sap that has been allowed to sour in painted, soldered, or terne-plated containers will also dissolve substantial amounts of lead.

DISCUSSION OF METHODS

In recent work by Wichmann and others[2] now included in the fourth edition of the *Methods of Analysis, A.O.A.C.*, various procedures are given for the determination of lead in food products. The one most adaptable to maple sirup from the standpoint of greatest possible accuracy of recovery appears to be the ashing method followed by dithizone extraction after removal of tin, and subsequent electrolytic isolation of lead (as the peroxide), which is finally determined iodometrically. The serious

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* A sample of sirup from a producer having all galvanised gathering and storage equipment but whose tin evaporator had numerous soldered joints and from 250 to 300 soldered patches was found to contain 0.022 grains/lb. of lead.

[1] Laboratory Experiments on the Source, Prevention and Removal of Lead Contamination of Maple Products. Mimeograph copy from U. S. Food and Drug Administration, Washington, D. C.

[2] *This Journal*, 17, 108 (1934); 18, 315 (1935).

interference of bismuth is not likely to be encountered in maple products.

The ashing procedure is necessarily slow and therefore is not suitable for routine work when large numbers of samples are to be run daily.

The partial digestion or "mush" procedure, also presented by Wichmann and others for the preliminary solution of the sample, has also been recommended by them for sirups, but it was found by the writer that an excessive quantity of chloroform-dithizone solution was required in order to secure the green negative lead end point. The digestion of sugar with nitric acid appears to produce reactive groups, which seem to combine with cyanide and destroy its property of preventing the formation of zinc and other metallic dithizone complexes. This accounts for the comparatively large amount of dithizone required and results in the presence of an excessive amount of organic matter in the final solution for electrolysis, which may coat the electrodes and interfere with the removal of lead from solution.

A modification of the partial digestion method was used by the writer in order to prevent the accumulation of organic matter (decomposed dithizone) in the electrolyte. The lead was removed from the lead-dithizone complex in chloroform solution by extraction with 1 per cent nitric acid. Recoveries in this manner seemed to be complete and were rapidly accomplished without the extraction of interfering organic matter. No interference was encountered from tin and the extraction of reasonable quantities of zinc did not affect the final results. When precaution was taken, direct electrolysis of the 1 per cent nitric acid lead extract did not produce interfering chloride formation from decomposed dissolved chloroform.[1]

TABLE 1.—*Lead recovery from 1% HNO_3 that had been refluxed with boiling $CHCl_3$*

INTERVAL	Pb ADDED TO HNO_3	Pb RECOVERED	
minutes	*mg.*	*mg.*	*per cent*
10	0.250	0.249	99.6
30	0.250	0.252	100.8
60	0.250	0.251	100.4

Chloroform is surprisingly inert in the presence of 1 per cent nitric acid, even when refluxed with it at its boiling point for periods as long as an hour. Chlorine ions sufficient to cause interference with lead removed electrolytically are not formed under these conditions. One per cent nitric acid in 110 cc. portions was refluxed on a boiling water bath with 50 cc. of chloroform for varying periods of time after which it was cooled, separated, and filtered. Known quantities of lead added to 100 cc. of this acid were recovered within experimental error, as shown in Table 1.

[1] *Methods of Analysis, A.O.A.C.*, 1935, 381, 16(b).

Only the faintest trace of chlorides (considerably less than 1 mg.) was detected with silver nitrate after an hour's refluxing. No chloride interference was noted during the analysis of 500 samples of maple sirup for lead by the described method.

The question of the extraction of manganese by dithizone arose because this metal will plate out electrolytically and give greater iodometric titrations than does lead. No manganese was carried through to the solution for electrolysis from sirups containing added known quantities of manganese and lead, as is shown in Table 2.

TABLE 2.—*Lead recovery from sirups containing added known quantities of manganese*

SIRUP	Mn ADDED	Pb ADDED	Pb RECOVERED	
No.	*mg.*	*mg.*	*mg.*	*per cent*
3	0.150	0.250	0.247	98.8
3	0.250	0.500	0.486	97.2
3	1.000	0.500	0.487	97.4

The modified method follows:

MODIFIED METHOD

Weigh 50 grams of sirup (25 grams is recommended if more than 0.3 mg. of Pb is present or if the Zn content is abnormally high) into a 500 cc. tall-form beaker, and add 15 cc. of lead-free HNO_3 and 50 cc. of water. Bring the mixture to a boil and allow to simmer on the hot plate for 10–15 minutes. Transfer the solution to a 500 cc. separatory funnel, rinsing the beaker thoroughly into the funnel and dilute to about 350–400 cc. Add 20 cc. of 25 per cent citric acid and neutralize to litmus with 28% NH_4OH, adding 1 cc. in excess. Cool, add 10 cc. of 10% KCN (more may be required in the presence of excessive Fe or Zn) and 15 cc. of 0.2% dithizone in $CHCl_3$. Stopper and shake the funnel thoroughly, inverting and releasing the pressure several times. Allow the mixture to separate and draw off the $CHCl_3$ layer into a 125 cc. separatory funnel containing 20 cc. of NH_4OH (1+199). If the $CHCl_3$ is deep red in color, continue the extraction with 0.2% dithizone until the dithizone solution shows a purple tint. (In some cases it may be necessary to start with 0.5% dithizone.) Then extract once with a 15 cc. portion of 0.1% dithizone and continue with 0.01% strength until two successive extractions yield the negative green color (4–5 extractions are usually sufficient). Make the color observations as the dithizone solution flows down the funnel stem. (Slight emulsions can be drawn off without harm into the smaller funnel.)

Discard the original aqueous solution and shake the combined extracts thoroughly. Allow the layers to separate completely and draw off the $CHCl_3$ layer into a 250–500 cc. separatory funnel containing 110 cc. of 1% HNO_3. Swirl the funnel to remove adhering $CHCl_3$ extract, allow to settle, and add to the acid. (Any small amount of emulsion present may be included.) Discard the ammoniacal layer.

Stopper the funnel and shake vigorously until the original green color of the dithizone is restored, then shake for an additional 3–4 minutes and allow the layers to separate. Draw off the dithizone layer, which may be reserved for the recovery of the $CHCl_3$. Filter the aqueous layer through a dry filter and pipet off 100 cc. for electrolysis.

Conduct the electrolysis directly on the 100 cc. volume according to Wichmann-

Clifford[1] using the small 1″×5/16″ rotating anode and a current of 75–85 milliamperes. Bring the temperature of the electrolyte up to 65°C. and add about 100 mg. of $K_2Cr_2O_7$, allowing a little time for volatilization of dissolved $CHCl_3$ before starting the current. Electrolyze for 15 minutes, wash, and titrate the electrode with 0.001 *N* thiosulfate from a micro buret reading in 0.01 cc.

$$\frac{(1.1\times\text{titration}-\text{blank})\times\text{factor}\times 7}{\text{Wt. of sample (grams)}}=\text{grains Pb/lb.}$$

RESULTS

Twenty-nine samples of maple sirup were analyzed in duplicate for their lead content by the ash method and also by the modified partial digestion method presented here. The results are shown in Table 3.

TABLE 3.—*Comparison of lead recoveries by ashing and modified methods*

SAMPLE NO.	DESCRIPTION	LEAD, GRAIN/LB.	
		ASH METHOD	MODIFIED METHOD
1	Standard Sirup	0.011 0.010	0.012
2	Standard Sirup	0.030 0.030	0.030
3	Standard Sirup	0.001 0.001	0.001
4	Standard Sirup	0.014 0.014	0.015
5	Standard Sirup	0.003 0.003	0.003
6	Standard Sirup	0.002 0.003	0.002
7	Standard Sirup	0.005 0.005	0.004
8	Standard Sirup	0.005 0.005	0.003
9	Standard Sirup	0.013 0.012	0.011
10	Standard Sirup	0.010 0.011	0.013
11	Standard Sirup	0.009 0.010	0.009
12	Standard Sirup	0.009 0.009	0.008
13	Standard Sirup	0.033 0.032	0.033
14	Standard Sirup	0.011 0.010	0.009
15	Standard Sirup	0.007 0.008	0.008

[1] *This Journal*, **18**, 316 (1935); *Methods of Analysis, A.O.A.C.*, 1935, 383, **19**, **20**.

TABLE 3.—(*Continued*)

SAMPLE NO.	DESCRIPTION	LEAD, GRAIN/LB.	
		ASH METHOD	MODIFIED METHOD
16	Standard Sirup	0.002 0.001	0.002
17	Standard Sirup	0.004 0.004	0.005
18	Standard Sirup	0.001 0.001	0.002
19	Standard Sirup	0.004 0.004	0.004
20	Standard Sirup	0.005 0.004	0.004
21	Standard Sirup	0.014 0.014	0.016
22	Standard Sirup	0.004 0.005	0.006
23	Standard Sirup	0.009 0.009	0.012
24	Standard Sirup	0.003 0.003	0.005
25	Beauce Sugar	0.048 0.048	0.055
26	Dark Sirup*	0.144 0.145	0.156
27	D11618—Dark N. Y.	0.017 0.017	0.015
28	F5011—Grade A. Vt.	0.004 0.006	0.004
29	F3173—100% Vt.	0 010 0.010	0.009
30	F3080—Condensed Canadian	—	0.012

* Sample not commercial grade.

The recovery of known quantities of lead in the presence of tin and zinc by the modified procedure is shown in Table 4. It is noted that small quantities of these contaminants do not interfere, but abnormally large

TABLE 4.—*Recoveries of added known quantities of lead in the presence of added known quantities of tin and zinc*

SIRUP	Pb ADDED	Sn ADDED	Zn ADDED	Pb RECOVERED	
	mg.	*mg.*	*mg.*	*mg.*	*per cent*
3	0.250	+++		0.252	100.8
3	0.325	5.0	10.0	0.323	99.5
3	0.400*			0.396	99.0
18	0.250	50.0		0.251	100.2
18	0.050	30.0		0.052	103.4

* Unknown to analyst until later.

quantities of zinc in the presence of an appreciable amount of lead produce low results. One sample to which had been added 0.5 mg. of lead and 25 mg. of zinc required ten extractions with strong 0.2 per cent dithizone and yielded a lead recovery of 83.8 per cent. This condition should be rarely encountered.

Before analysis maple sugar should be reconstituted into sirup with known amounts of water and heated after the addition of about 1 per cent of concentrated nitric acid in order to liberate precipitated lead and distribute it uniformly in the sample. A comparatively large amount of lead may occur locally in solid or semi-solid maple products and ordinary mixing by grinding in a mortar is insufficient to distribute it evenly.

The writer is deeply indebted to W. B. White and H. J. Wichmann of the U. S. Food and Drug Administration, Washington, D. C., for their constructive criticisms of the manuscript.

CONCLUSIONS

A modified partial digestion method for the determination of lead in maple products is suggested.

With the proper apparatus and assistance about twenty-five samples can be run daily. A single sample can be reported out in about two hours.

Tin, manganese, and reasonable amounts of zinc do not interfere with the lead recovery, as was indicated from the analysis of sirups containing added known quantities of lead plus normal amounts of these contaminants.

During the 1937 season in Vermont and New York state control laboratories the lead content of about 1000 sirups was determined by the method described with no apparent difficulty.

OCCURRENCE AND DETERMINATION OF ZINC IN MAPLE PRODUCTS

By J. L. Perlman and J. E. Mensching (State Food Laboratory, New York State Department of Agriculture and Markets, Albany, N. Y.)

The diphenylthiocarbazone (dithizone) methods used recently for the determination of contaminant lead in maple products indicate that zinc is also likely to be present in considerable amounts. Increasing recognition of the toxicity and the common occurrence of this metal makes necessary the availability of a dependable micro method for its accurate determination.

The principal sources of zinc contamination in maple products are galvanized equipment and equipment that has been coated with paints bearing zinc pigment, such as sap-gathering utensils, and hauling and storage tanks. Solution of the metal from these galvanized or coated containers is hastened by the prolonged boiling necessary for the concentration of the

sap, as well as by the acidity due to incipient and progressed fermentation, or "souring."

Methods for the determination of small quantities of zinc in foods and biological materials in the presence of the interferences commonly occurring in maple products are scarce. Ashing has been employed for sample preparation by Sylvester and Hughes,[1] but it is rather tedious and the possibility of erroneous recoveries due to volatilization as well as to the difficulty of eliminating inteferences has discouraged its use.

It was found by the writers that under controlled conditions zinc can be satisfactorily extracted from maple products with dithizone in chloroform. As other metals are extracted under the same conditions, the employment of a highly selective step for the final determination is necessary.

Lang[2] evolved an iodometric method for the determination of zinc in slightly acid solution by the use of potassium ferricyanide. Hibbard[3] confirmed Lang's findings, as did Sahyun and Feldkamp later,[4] and applied the method successfully to soil and biological materials. The reaction involved is expressed as follows:

$$2K_3Fe(CN)_6+2KI+3ZnSO_4\rightarrow K_2Zn_3[Fe(CN)_6]_2+3K_2SO_4+I_2.$$

In this investigation the Lang method was modified and applied to the dithizone extract obtained from the partially digested or "mushed" maple sample. Sample preparation is quickly and easily accomplished, allowing ready adaptation of the method to routine work. Dithizone extraction is carried out in weak ammoniacal solution in contrast to the acid medium (pH 4.5) used by Sylvester and Hughes. The acid procedure offers no advantage over the ammoniacal since in either case other metals extracted with the zinc give no interference in the final determination. Sylvester and Hughes also utilize a modification of Lang's method for final zinc titration, but their entire procedure is rather long and it is not readily adaptable to routine work.

The writers found that metallic contaminants commonly present offered no serious interferences and recoveries were within experimental error. The method used follows:

METHOD

Weigh 25 grams of sirup (sugar should be reconstituted into sirup) into a 500 cc. tall-form beaker, and add 50 cc. of distilled water and 10 cc. zinc-free HNO_3. (A 50 gram sample should be used if less than 0.1 mg. of Zn is present.) Mix, and boil on a hot plate for 15–20 minutes, keeping the beaker covered with a watch-glass. Transfer the solution to a 500 cc. separatory funnel and dilute to 350–400 cc. with distilled water. Add 20 cc. of 25% citric acid and neutralize to litmus with strong ammonia, adding not more than 0.5 cc. in excess. Cool, add 15 cc. of 0.2% dithizone in $CHCl_3$, and shake vigorously until the dithizone no longer changes color.

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[1] *Analyst*, **61**, 734 (1936).
[2] *Z. anal. Chem.*, **79**, 161 (1929); **93**, 21 (1933).
[3] *Ind. Eng. Chem. Anal. Ed.*, **6**, 423 (1934).
[4] *J. Biol. Chem.*, **116**, 555 (1936).

Allow the $CHCl_3$ layer to settle and draw it off into a 125 cc. separatory funnel containing 20 cc. of ammonia (1+999). If the $CHCl_3$ layer, as it runs down the funnel stem, is red in color, re-extract with another 15 cc. portion of the 0.2% strength. Repeat with this strength if necessary until a blue to purple color is obtained. Continue extracting with 0.1% dithizone and finally with 0.01% strength until two consecutive extracts show the negative green color. Test the original solution with litmus between extractions to make certain it remains ammoniacal, adding 1–2 drops of strong ammonia if necessary. Discard the original solution. Shake the combined extracts vigorously with the dilute ammonia, allowing the layers to separate completely, and carefully draw off the dithizone $CHCl_3$ into another 125 cc. separatory funnel containing 25 cc. of 1% HNO_3 accurately measured from a pipet or buret. Swirl the funnel to remove adhering extract, which is allowed to settle and drawn off into the acid funnel. (A small amount of emulsion, if present, can be included.) Shake the dithizone extract vigorously with the dilute acid until the original green color is restored, after which continue to shake for an additional 3–4 minutes. After the layers separate, draw off the dithizone $CHCl_3$ and reserve for $CHCl_3$ recovery according to Biddle.[1] Pass the acid layer through a small dry filter, and evaporate 20 cc. of the clear filtrate to dryness in a 200 cc. tall-form beaker in a boiling water bath.

Add 2–3 cc. of alcohol to the dry residue and carefully bring to a boil on a hot plate. When maximum solution of the organic matter is effected, slowly add 5 cc. of $0.02N$ H_2SO_4, washing down the sides of the beaker. Again bring the solution to a boil and continue to boil gently while swirling for about 1 minute. Add 10 cc. of distilled water, gently boil for 3–5 minutes, and allow to cool to room temperature.

Titrate the zinc as follows: Add 1 cc. of 0.5% starch and 1 cc. of fresh 10% KI, mix and allow the solution to stand for about a minute. If iodine is liberated at this point due to the presence of metals other than zinc, add dropwise 0.001 N $Na_2S_2O_3$ until the starch iodide color is discharged. Add 1 cc. of freshly prepared $K_3Fe(CN)_6$, stir the solution, and allow to stand for 3 minutes. Titrate the iodine liberated against 0.001 N $Na_2S_2O_3$ from a micro buret until the addition of 1 drop does not cause any change in the yellowish color at the point of contact with the liquid, using a white background. 1 cc. of 0.001 N $Na_2S_2O_3$ = 0.1 mg. of zinc. Determine a blank for the reagents used.

Amounts of zinc as low as 0.05 mg. can be recovered from maple products by the outlined procedure. The final acid extract should be so divided into aliquot parts as to give not more than 1 mg. of zinc for titration (10 cc. $Na_2S_2O_3/1000$).

cc. 0.001 N $Na_2S_2O_3 \times \text{factor} \times 0.1 \times 1000$/wt. of sample represented in aliquot in grams = p.p.m. of Zn.

DISCUSSION

No difficulties were encountered with this method when it was used in the presence of lead, tin, copper, bismuth, and thallium salts; 1 mg. of cadmium as $CdCl_2$ yielded a titration of 0.85 cc. of 0.001 N sodium thiosulfate, but after purification of the salt this was reduced to 0.25 cc., indicating the presence of active impurities. Copper combines with dithizone under the conditions of the method, forming a very stable complex which is not broken up by 1 per cent nitric acid.

Known quantities of zinc were determined satisfactorily in the presence of lead and tin, as shown in Table 1.

[1] *Ind. Eng. Chem. Anal. Ed.*, **8**, 99 (1936).

TABLE 1.—*Recovery of Zn in the presence of Pb and Sn*

	Zn ADDED	Pb ADDED	Sn ADDED	Zn RECOVERED
	mg.	*mg.*	*mg.*	*mg.*
1	0.250	0.50	—	0.262
2	1.000	0.50	—	1.004
3	0.750	—	0.50	0.748

Recoveries of known quantities of zinc from aqueous solutions and maple sirup are shown in Table 2.

TABLE 2.—*Recoveries of known quantities of Zn*

SAMPLE	Zn ADDED	Zn RECOVERED
	mg.	*mg.*
Aqueous Solution No. 1	0.500	0.499
2	0.500	0.492
3	0.500	0.495
4	0.250	0.259
5	0.750	0.739
6	1.000	1.000
Sirup No. 361*	—	0.183
Sirup No. 361	0.500	0.478
Sirup No. 361	0.750	0.720
Sirup No. 361	1.000	0.963
Sirup No. 361	1.250	1.192

* Less blank of 0.183 mg.

The zinc content of 54 samples of standard maple sirup was determined. The results are given in Table 3. It might be well to note that during analysis none of these samples showed visible liberation of iodine before the addition of potassium ferricyanide.

TABLE 3.—*Zn contents of standard maple sirup*

SAMPLE NO.	Zn	SAMPLE NO.	Zn
	p.p.m.		*p.p.m.*
329	31.2	356	55.1
330	55.9	357	23.7
331	23.4	358	31.5
332	8.8	359	44.2
333	71.7	360	9.4
334	18.5	361	8.1
335	17.8	362	84.0
336	67.6	363	10.9
337	56.0	364	42.6
338	44.4	98	422.2
339	37.2	277	255.8
340	15.2	295	204.9
341	68.9	298	51.5
342	66.8	299	217.4

TABLE 3.—(*Continued*)

SAMPLE NO.	Zn	SAMPLE NO.	Zn
343	8.5	302	30.7
344	9.3	305	116.0
345	23.5	313	70.7
346	61.1	316	36.4
347	34.3	318	246.5
348	24.4	319	39.5
349	53.3	326	96.2
350	114.9	228	57.5
351	61.1	274	207.9
352	16.9	212	13.8
353	9.6	170	20.3
354	45.8	268	47.1
355	27.6	304	20.8

SUMMARY

A method for the micro determination of contaminant zinc in maple products is described.

Values for the zinc content of 54 samples of standard maple sirup are given.

THE ANALYSIS OF AMINOPHYLLINE

By F. REIMERS (Laboratory of the Danish Pharmacopoeial Commission, Copenhagen, Denmark)

The substances that should be determined in aminophylline are theophylline, ethylene-diamine, and water. In "New and Non-official Remedies, 1936" theophylline is determined iodometrically, water by drying for 48 hours over calcium chloride and ethylene-diamine (together with theophylline) by the Kjeldahl method. The U.S.P. XI has no method for ethylene-diamine; determines water by drying over sulfuric acid for 48 hours; and determines theophylline by means of a combination of precipitation by acidification, conversion of the nonprecipitated alkaloid into caffeine, and extraction of the caffeine.

This paper shows that the methods quoted need revision in certain respects, and that the determination of the composition of aminophylline can be simplified.

I. DETERMINATION OF THEOPHYLLINE

Besides the iodometric determination of theophylline and the conversion into caffeine by the quantitative methylation method of Self and

Rankin,[1] an argentometric titration was used. A critical examination of several modifications of these three methods with theophylline-sodium-acetate by the writer[2] showed that the argentometric method gives high results, probably owing to an adsorption of silver nitrate by the highly voluminous theophylline-silver. The iodometric method gives low results, and they vary greatly with the excess of iodine, but it is only by using excesses of iodine (150 per cent and more), that comparatively correct results can be obtained. The method of Self and Rankin on the other hand, gives correct results. It proved possible, however, to leave out the methylation of the theophylline by using for the extraction a mixture of one volume of isopropyl alcohol and three volumes of chloroform, instead of chloroform alone. Although theophylline is less soluble in chloroform than in water, and is only slightly more soluble in isopropyl alcohol, mixtures of these two solvents dissolve 8–9 times more than does water, so that these mixtures can be used for direct extraction of theophylline, similar to the extraction of caffeine by chloroform.

TABLE 1.—*Solubility of theophylline in mixtures of chloroform and isopropyl alcohol at 20°C.*

CHLOROFORM	ISOPROPYL ALCOHOL	CRYSTALLINE THEOPHYLLINE DISSOLVED IN 100 G. OF SOLVENT	SOLUBILITY
cc.	*cc.*	*grams*	
100	0	0.32	1+313
80	20	3.26	1+ 30.7
75	25	4.18	1+ 23.9
67	33	4.92	1+ 20.3
60	40	4.88	1+ 20.5
50	50	4.60	1+ 21.7
25	75	2.42	1+ 41.3
0	100	0.65	1+154

The extraction of theophylline from aminophylline is made as follows: 0.300 gram of aminophylline is dissolved in a small separator in 2 cc. of water; 2 drops of methyl red indicator are added and then dilute hydrochloric acid to color change. Extraction is continued for 1 minute with 25 cc. of a mixture of three volumes of chloroform and 1 volume of isopropyl alcohol, and the extract is filtered through a small filter into a weighed flask. The extraction is repeated three times with 20, 20, and 10 cc., respectively, of the same solvent, all the extracts being filtered through the same filter into the flask, and afterwards evaporated on the water bath to dryness. The flask with the contents is now dried to constant weight at 100°C. The weight of anhydrous theophylline×1.100 (log. 0.04141) = the weight of the corresponding quantity of crystalline theophylline.

[1] *Quart. J. Pharm. Pharmacol.*, **4**, 346 (1931).
[2] *Dansk Tids. Farmaci*, **9**, 11 (1935).

It proved necessary to mix the aminophylline carefully before weighing, as the substance was not in all cases quite homogeneous. Thus the analysis of a sample of unmixed substance showed a percentage of theophylline varying from 68.5 to 71, while the analysis of equal quantities of a solution of larger quantities of the same substance showed 69.0, 69.1, and 69.0 per cent (after the method of "New and Non-official Remedies": 68.5 per cent). Some results of the extraction method applied to known quantities of theophylline dissolved in ethylene-diamine solution are shown in Table 2. These results by the separator method are entirely satisfactory, while somewhat lower results are obtained by using the method of Schulek and Szeghö[1] for the analysis of alkaloid salts. The substance is here dissolved in a small quantity of water and shaken with a solvent, the water being removed by addition of dried sodium sulfate.

TABLE 2.—*Theophylline in a solution of theophylline in ethylene-diamine solution by extraction with chloroform-isopropyl alcohol (3 vols. +1 vol.)*

THEOPHYLLINE	EXTRACTION METHOD	SOLVENT USED	THEOPHYLLINE FOUND
gram		*cc.*	*per cent*
0.2500	Schulek	50+10+10	99.8, 99.0, 98.8
0.2500	Schulek	40+10+10+10	99.5, 99.2, 100.5
0.2500	Separator	25+25+25	98.8, 99.0, 98.8
0.2500	Separator	25+20+20+10	100.1, 100.2, 100.3
			99.8, 100.1, 100.5*

* Extract filtered through cotton.

The method given for the isolation of theophylline can also be used for the determination of theophylline in tablets and in other soluble theophylline compounds, as theophylline-sodium acetate. In dealing with the latter substance, however, it is necessary to remove the acetic acid before the extraction, as the results will otherwise be too high. This is easily done on a water bath by a triple evaporation of the substance, together with a small quantity of dilute sulfuric acid and water. By this method it is possible to extract theophylline, not only from the slightly acid solution, as was done in the analysis of aminophylline, but also from solutions containing a surplus of acid, or from slightly alkaline solutions containing *e.g.*, ammonia.* In this case, however, the transfer of theophylline from the watery solution to the chloroform-isopropyl alcohol layer is less complete, so that it will be necessary to repeat the extraction six to seven times.

DETERMINATION OF ETHYLENE-DIAMINE

An electrometric titration of a solution of ethylene-diamine "Merck" showed that this substance can be titrated exactly as a bivalent base, with 0.1 N hydrochloric acid to pH of approximately 4.6. In good agreement with this, it is found that methyl red does not give a well-defined

[1] *Pharm. Zentr.*, **72**, 497 (1931).
* According to unpublished investigations by K. A. Jackerott.

color change, while methyl orange and bromphenol blue give good color changes, but produce results a little too high unless the titration is stopped at the beginning of the color change. The best way of titrating to pH 4.6 is by using bromcresol green for indicator and titrating to a green color. Table 3 shows the results of a series of titrations with different indicators. The figures given represent the average results of several titrations. The table further shows that the result is not influenced by the presence of theophylline.

TABLE 3.—*Titration of ethylene-diamine (10 cc. of a solution of about 2 grams of ethylene-diamine in 250 cc. of water)*

INDICATOR	COLOR OF INDICATOR	THEOPHYLLINE	0.1 N HCl
		gram	*cc.*
Methyl red	Orange	0	18.80
Methyl red	Red	0	19.0–19.1
Methyl orange	Orange	0	19.17
Bromphenol blue	Bluish green	0	19.16
Bromcresol green	Green	0	19.11
Bromcresol green	Greenish yellow	0	19.15
Bromcresol green	Green	0.20	19.10

DETERMINATION OF WATER

The weight of a sample of aminophylline, dried over calcium chloride, did not become constant until after 3 days' drying. The loss of weight (7.8 per cent) was due to loss of water and also of ethylene-diamine. A titration showed an evaporation of 2.1 per cent of the latter and 5.7 per cent of the former substance. Ethylene-diamine evaporates on exposure to air. In 3 hours three different preparations lost 0.7, 1.5, and 2.1 per cent, respectively. The evaporation became slower, so that after a week the losses were 1.2, 3.1 and 3.0 per cent. Therefore it is not possible to obtain a correct determination of water by drying alone, while it is possible by drying at a higher temperature, for example, 125°C., to make not only water but also ethylene-diamine evaporate completely in the course of a few hours. At 105°C. the drying proceeds somewhat more slowly, and must be continued for 24 hours.

After water and ethylene-diamine have been removed by drying at 125°C., for example, only theophylline should be left, and therefore this

TABLE 4.—*Analysis of aminophylline*

PREPARATION	CRYSTALLINE THEOPHYLLINE		ETHYLENE-DIAMINE	ETHYLENE-DIAMINE +WATER
	EXTRACTION	DRYING AT 125° C.		
	per cent	*per cent*	*per cent*	*per cent*
A	79.9	80.2	15.9	27.1
B	86.0	86.0	14.4	21.8
C	80.4	80.9	17.4	26.5

procedure can be used as a simple and rapid determination of theophylline, when combined with an examination of the purity of the theophylline. Table 4 shows the results of examinations of three preparations of aminophylline. The percentages of theophylline obtained by extraction and by drying agree very well.

SUMMARY

(1) Theophylline can be determined quantitatively in aminophylline by extraction with a mixture of three volumes of chloroform and one volume of isopropyl alcohol, the solution being slightly acidified. This extraction may be applied to tablets and to other preparations containing theophylline.

(2) The ethylene-diamine in aminophylline may be titrated by using standardized acid with bromcresol green as indicator (pH about 4.6).

(3) In drying at ordinary temperature the loss in weight is due to evaporation of both ethylene-diamine and water. These two substances can be removed completely by drying at 125°C. Consequently this drying, in connection with a titration of ethylene-diamine, can be used for determining the percentage of water, and, in connection with an examination of the purity of the theophylline, for determining the percentage of theophylline.

RAPID DETERMINATION OF IRON IN PHARMACEUTICALS WITH THE AID OF CUPFERRON AND AN IMMISCIBLE SOLVENT*

By S. M. Berman, J. J. Chap, and D. M. Taylor (U. S. Food and Drug Administration, Philadelphia, Pa.)

The use of the ammonium salt of nitrosophenylhydroxylamine, commonly known as cupferron, as a quantitative precipitant for iron was suggested by Baudisch.[1] Its application to the determination of iron in solutions of organic matter was studied by Ferrari.[2] A statement by Baudisch and King[3] to the effect that the iron precipitate with cupferron could be dissolved in various organic solvents indicated the possibility that an extraction method specifying an immiscible solvent such as chloroform might be feasible. After considerable preliminary work by the writers, the following procedure, which appears to be generally applicable to pharmaceutical preparations of iron, was developed.

RECOMMENDED PROCEDURE

To the solution, in a pear-shaped separator containing not more than 100 mg. of Fe, add sufficient HCl to make the total acidity equivalent to at least 10 cc. of 4 normal. Add 6% aqueous solution of cupferron until the reagent produces a momentarily white precipitate at the point of contact with the acid solution. Add

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* Read before the Drug Section of the Association at the Annual Meeting, December, 1936.

1 *Chem. Ztg.* **33**, 1298 (1909); **35**, 913 (1911); *Analyst*, **35**, 78 (1910); **36**, 520 (1911).

2 *Ann. chim. applicata*, **4**, 341 (1915); *J. Ind. Eng. Chem.*, **12**, 345 (1920).

3 *J. Ind. Eng. Chem.*, **3**, 629 (1911).

10 cc. of $CHCl_3$, or more, and shake the contents of the separator vigorously for 15 seconds, or more gently for a longer period, according to the degree of facility of separation expected from the two liquids. When the layers have separated, again add the reagent until the white precipitate reappears. (In the presence of any iron the initial white precipitate quickly changes to pinkish brown.) Repeat the shaking-out process, without removing the $CHCl_3$ layer, until after alternate treatment with reagent and shaking out the aqueous layer is quite clear of any turbidity but that due to suspended $CHCl_3$. Draw off the clear $CHCl_3$ layer into a second separator, and extract the aqueous solution in the first separator with small portions of $CHCl_3$ until the solvent remains colorless, adding these extracts to the extract in the second separator. Wash the combined $CHCl_3$ extracts with about 10 cc. of H_2O and draw off into a weighed dish suitable for ignition.

Evaporate the solvent on a steam bath, and heat the residue at 150°–300°C. under a good hood until fuming ceases. Ignite at 500°–700°C. to constant weight, cool, and weigh as Fe_2O_3; or determine the iron volumetrically by the method of Zimmerman-Reinhardt, as defined by Jones and Jeffery.[1]

EXPERIMENTAL

The method described was applied to solutions of ferrous and ferric iron containing no organic matter and to solutions of ferrous and ferric

TABLE 1.—*Description of possible interferences in iron assay*

PRODUCT	SAMPLE SIZE	INTERFERENCE
	cc.	
Tincture Ferric Citrochloride NF	1.5	450 g./liter Na citrate
Soln. Iron & Ammonium Acetate NF	(A) 30.0	9.5 cc./liter acetic acid
	(B) 50.0	120 cc./liter glycerol
		37.5 g./liter NH_4 acetate
		38 g./liter sucrose
Elixir Gentian & Iron NF	15.0	72 g./liter Na citrate
		192 g./liter sucrose
		45 cc./liter glycerol
Sirup Ferrous Iodide USP	10.0	60 g./liter iodine
		850 g./liter sucrose
Sirup Hypophosphites Comp. NF	100.0	35 g./liter Ca hypophosphite
		17.5 g./liter K hypophosphite
		17.5 g./liter Na hypophosphite
		2.2 g./liter Mn hypophosphite
		1.1 g./liter Quinine hypophosphite
		3.7 g./liter Na citrate
		700 g./liter sucrose
		50 cc./liter glycerol
Elixir Iron, Quinine & Strychnine NF	20.0	8.2 g./liter alkaloid salt
		56 g./liter Na citrate
	gram	300 cc./liter glycerol
Capsules Glycerophosphates Comp.	10.4	86% Ca glycerophosphate
		6% Fe glycerophosphate
		4% Mn glycerophosphate
		4% Quinine glycerophosphate
Iron and Ammonium Citrates USP	0.5	about 80% citrate

[1] *Analyst*, 34, 306 (1909).

salts that contained in addition varying amounts of organic substances in common use for pharmaceutical and therapeutic purposes. Several of the preparations that are official in the United States Pharmacopoeia or the National Formulary were compounded in the laboratory, the remainder being commercial products, as were the non-official specialties. In Table 1 are given the names of the various pharmaceuticals used, the portions actually analyzed, and the substances present that might be expected to interfere with the usual iron methods. All the materials were liquids or water-soluble solids.

TABLE 2.—*Cupferron extraction: comparison with ignition methods for Fe*

SUBSTANCE		CUPFERRON EXTRACTION GRAVIMETRIC	CUPFERRON EXTRACTION VOLUMETRIC	IGNITION VOLUMETRIC	NO TREATMENT U.S.P. XI
		gram	*gram*	*gram*	*gram*
Ferrous Ammonium Sulfate		0.1393	—	0.1398	
				0.1398	
Solution Ferric Chloride USP			0.1420	0.1407	
Tincture Ferric Chloride USP		—	0.0746	0.0743	
		—	0.0748		
Tincture Ferric Citrochloride NF		0.0749	0.0753		
Brown Fe & Amm. Citrate NF		0.0867	0.0868	0.0868	
		0.0871	—		
Green Fe & Amm. Citrate NF		0.0789	0.0790		0.0782*
Soln. Fe & Amm. Acetate NF	(A)	0.0590	0.0588		
	(B)	0.0992	0.0994		
Elixir Gentian & Iron NF		0.0757	0.0754	0.0759	
		0.0758	0.0754		
Sirup Ferrous Iodide USP		—	0.1213		0.1208†
Sirup Ferrous Sulfate		—	0.1052	0.1050	
Sirup Hypophosphites Comp. NF		—	0.0475	0.0466	
Capsules Glycerophos. Comp.		—	0.859%	0.865%	
Elixir Fe, Quin., Strych. NF	(A)	0.0626	—	0.0626	
		—	0.0623	0.0626	
	(B)	0.0593	0.0593	0.0590	
		0.0591	0.0591	0.0592	
		—	0.0591	0.0593	

* Iodometric titration.
† Argentometric titration.

The results obtained are given in Table 2. The agreement between the various procedures is believed to be satisfactory for the most part, such discrepancies as exist being ascribed to the fact that only one experiment was made in each particular case. For instance, the ashing of compound sirup of hypophosphites is a relatively long and arduous undertaking when a 100 cc. sample is required. This preparation is also one of the most difficult to extract, approximately one hour being required by the method described.

While it was theoretically possible to demonstrate the completeness of extraction by testing the aqueous residue for iron, this would be a burden-

some task with such materials as the glycerophosphates compound and the sirup of hypophosphites compound. It appeared to be sufficient to compare the results by the cupferron extraction with results obtained on the same type of substance from which the organic matter had been removed before determining the iron. In all cases ashing methods were used to destroy the organic matter, and collateral tests were made to assure absence of error in the final permanganate titration, as might ensue from the presence of reduced phosphorous compounds. As a further check, after obtaining the weights of the residues the writers titrated the ignited cupferron extraction residues as they had the controls, using tared 150 cc. beakers for the ignition. The employment of tared glass beakers (weighed up against beakers of similar material and volume plus small weights) is considered by the writers to be a convenient and satisfactory arrangement. In some cases no weighing was made. However, the results (Table 2) include sufficient instances where both the weight and the permanganate titer were obtained. As a matter of interest there are included an assay of sirup of ferrous iodide by the argentometric titration official in U.S.P. XI, which does not require removal of organic matter, and an assay of green iron and ammonium citrate by the iodometric method official in U.S.P. XI, which is also not supposed to require removal of the organic matter.

SUMMARY

(1) A new method of employing cupferron is described; it depends upon the use of an immiscible solvent to extract the iron salt precipitated by this reagent.

(2) The method was applied to twelve common pharmaceutical preparations containing iron, and a tabulation of results is given.

(3) Results obtained by the new method are compared with results by a standard method, on the same material, and shown to be satisfactory.

THE DETERMINATION OF SMALL QUANTITIES OF STRYCHNINE

By A. G. Murray (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

Strychnine is ordinarily determined by dissolving the separated alkaloid in an excess of standard acid and titrating the excess with standard alkali, methyl red being used as an indicator. The method is entirely satisfactory when a sufficient amount of strychnine is available. The assay process for tablets of strychnine sulfate in The National Formulary VI (p. 391) specifies the use of sufficient material to yield about 130 mg. of the salt. The average dose of strychnine sulfate, as stated in the Pharmacopoeia and in The National Formulary, is 2 mg. The assay process therefore contemplates the use of approximately 65 average medic-

inal doses for a single determination. A method that employs a smaller quantity of the alkaloid would often be advantageous.

Gordin and Prescott[1] proposed an iodometric method for the determination of several alkaloids including strychnine. They reported that under specified conditions Wagner's reagent (iodine-potassium iodide solution) yields with strychnine in acid solution a precipitate having the composition $C_{21}H_{22}N_2O_2 \cdot HI \cdot I_6$. By using a measured excessive volume of the standardized reagent and titrating an aliquot part of the filtrate they reported results agreeing closely with the amount of strychnine used. Because of the large consumption of iodine in the reaction and the delicacy of the end point in the thiosulfate titration the method appeared to be well suited for the determination of strychnine, particularly when small quantities are involved.

Attempts to apply the suggested procedure resulted in disappointment. Conditions under which a precipitate of constant composition would result were not found. Varying the composition and amount of the reagent, the concentration of the strychnine solution, the order in which the solutions are mixed, and the time of reaction resulted in precipitates containing varying proportions of iodine, in some instances considerably more, in others less than would correspond to the formula assigned by Gordin and Prescott. The precipitate was always amorphous. The idea of using this reaction as a quantitative method for strychnine was reluctantly abandoned.

As early as 1862, Mayer[2] proposed the use of potassium mercuric iodide for the determination of several alkaloids, including strychnine, and since the publication of his article, this reagent, which previously had been used for the detection of alkaloids, has been known by his name. It yields with acid solutions of strychnine a highly insoluble precipitate, $C_{21}H_{22}N_2O_2 \cdot HI \cdot HgI_2$, amorphous at first, but soon becoming crystalline.

Mayer simply titrated a solution of an alkaloid in dilute acid with a standard potassium mercuric iodide solution until no further precipitation occurred. He evidently recognized the difficulties and limitations of a method using as an end point the absence of further precipitation, as well as the desirability of a procedure depending upon the determination of iodide in the filtrate, as he states "I have as yet not been successful in using for a test-method the iodine remaining in solution." It is the accomplishment of this object, in so far as its application to the assay for strychnine is concerned, that is reported in this paper.

In a subsequent paper, Mayer[3] proposed to determine the excess iodine (and chlorine) in the filtrate by titration with standard silver nitrate solution. Heikel[4] modified Mayer's process by determining the mercury in the filtrate.

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[1] *J. Am. Chem. Soc.*, **20**, 706 (1898)
[2] *Proc. Am. Pharm. Assn.*, **10**, 238 (1862).
[3] *Am. J. Pharm.*, **35**, 20 (1863).
[4] *Chem. Ztg.*, **32**, 1149, 1162, 1186, 1212 (1908).

The simplicity and accuracy of the titration of iodide with potassium iodate solution described by Andrews[1] and the sharpness of the end point suggested its use in determining the iodide in the filtrate from the strychnine hydriodide-mercuric iodide precipitate. The method was found serviceable for as little as 5 mg. of strychnine.

The details recommended are as follows:

METHOD

REAGENTS

(a) *Potassium mercuric iodide solution.*—Dissolve 0.46 gram of HgI_2 and 0.40 gram of KI (or 0.27 gram of $HgCl_2$ and 0.73 gram of KI) in a little water and dilute to 100 cc. (This solution contains approximately 0.01 millimole of mercuric iodide and 0.024 millimole of potassium iodide per cc. The excess of potassium iodide over the theoretical proportion for the formation of K_2HgI_4 is necessary to prevent precipitation of a small amount of mercuric iodide in so dilute a solution. If, upon standing, red crystals of mercuric iodide separate, they should be filtered off and 1 or 2 mg. more potassium iodide added to the filtrate. Standardize this solution against reagent (b) or make a blank determination as directed below. Protect the reagent from light.

(b) *Potassium iodate solution.*—0.01 *M*. Dissolve 1.0701 grams of reagent-grade potassium iodate, previously dried at 120°, in sufficient 1% HCl to make 500 cc. The acid will keep the solution free from mold.

DETERMINATION

Dissolve the strychnine in 2 or 3 cc. of dilute sulfuric or hydrochloric acid and transfer to a 25 cc. graduated flask. Add an accurately measured volume of the HgI_2-KI solution, at least 1 cc. for each 3 mg. of strychnine. Dilute to the mark. Thoroughly mix the contents of the flask and let stand, protected from light, for 1–2 hours. Filter, rejecting the first 2 or 3 cc. of the filtrate. Transfer 20 cc. of the filtrate to a glass-stoppered bottle. (A 50 cc. separator is appropriate.) Add about 16 cc. of concentrated (35–37%) HCl. Cool. (The process may be facilitated by cooling the acid and the measured portion of the filtrate in an ice bath before mixing.) Add about 1.5 cc. of $CHCl_3$ and titrate with the standard iodate solution until the iodine at first set free has been converted into ICl, as indicated by the disappearance of color from the $CHCl_3$. Toward the end of the titration shake the mixture vigorously after each addition of iodate. (The titration can be more conveniently conducted by postponing the addition of the $CHCl_3$ until the end point is almost reached. The end point of the titration cannot be accurately determined by observing the disappearance of color from the aqueous solution since the ICl gives it a yellowish tint.) Since the end point is easily observed within 0.02 cc. of 0.01 *M* iodate, a narrow buret (10 cc.) should be used for accurate reading. The HCl content of the aqueous liquid must be maintained at not less than 12% throughout the titration by adding concentrated acid if necessary. This is conveniently effected by maintaining the volume of concentrated acid in the solution at not less than one-third of the total volume.

Make a blank determination, using the same volume of the HgI_2-KI solution. From these data calculate the volume of iodate solution corresponding to the iodide combined with the strychnine.

1 cc. of 0.01 *M* KIO_3 is equivalent to—

2.228 mg. of strychnine ($C_{21}H_{22}N_2O_2$).
2.648 mg. of strychnine nitrate ($C_{21}H_{22}N_2O_2 \cdot HNO_3$).
2.855 mg. of strychnine sulfate ($(C_{21}H_{22}N_2O_2)_2 \cdot H_2SO_4 \cdot 5H_2O$).

[1] *J. Am. Chem. Soc.*, **25**, 756 (1903).

It is, of course, essential that a sufficient volume of the reagent to supply an excess of mercury be used for the precipitation of the strychnine. In case of doubt, test the filtrate for mercury. The presence of excess iodide is no evidence that there is also an excess of mercury.

The degree of accuracy attainable by the method presented is indicated by the following data:

Finely crystallized strychnine (1.0015 grams), previously dried over calcium chloride overnight, was dissolved in 10 cc. of 5 per cent sulfuric acid, and the solution was diluted to 1 liter. Measured volumes of this solution were placed in 25 cc. graduated flasks and diluted with 10–15 cc. of water; 3 cc. of the potassium mercuric iodide reagent was added, and the determination was carried out as described previously. The data are given in Table 1. The relatively high percentage errors observed for amounts of strychnine below 4 mg. are probably not significant since experimental errors in the measuring of the mercuric reagent or in the iodate titration might readily account for them.

TABLE 1.—*Results on strychnine*

STRYCHNINE TAKEN	HgI_2-KI SOLN	20 ML. FILTRATE REQUIRES KIO_3 SOLN	KIO_3 SOLN EQUIV. TO STRYCHNINE IN 20 ML. FILTRATE	CALC'D KIO_3 SOLN EQUIV. TO TOTAL STRYCHNINE	STRYCHNINE FOUND	ERROR
mg.	*cc.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*
			Series (a)			
0.00	3.00	4.99				
4.01	3.00	3.49	1.50	1.88	4.19	+ 4.5
5.01	3.00	3.15	1.84	2.30	5.12	+ 2.2
6.01	3.00	2.77	2.22	2.78	6.19	+ 3.0
7.01	3.00	2.42	2.57	3.21	7.15	+ 2.0
9.01	3.00	1.73	3.26	4.08	9.09	+ 0.9
			Series (b)			
0.00	3.00	9.02				
1.00	3.00	8.62	0.40	0.50	1.11	+11.0
2.00	3.00	8.25	0.77	0.96	2.14	+ 7.0
3.00	3.00	7.90	1.12	1.40	3.12	+ 4.0
4.01	3.00	7.53	1.49	1.86	4.14	+ 3.5
5.01	3.00	7.22	1.80	2.25	5.01	0.
6.01	3.00	6.80	2.20	2.75	6.13	+ 2.0
8.01	3.00	6.15	2.87	3.59	8.00	0.
9.01	3.00	5.75	3.27	4.09	9.11	+ 1.1

EFFECT OF ACID CONCENTRATION ON PRECIPITATION

To 5 cc. aliquots (containing 5.01 mg. of strychnine) of the previously described strychnine solution were added varying volumes of concentrated hydrochloric acid and water, followed by the addition of 3 cc. of the reagent. The volumes were completed to 25 cc. and treated as described previously.

TABLE 2.—*Effect of acid on precipitation*

CONC. HCl	WATER	20 ML. FILTRATE REQUIRES KIO_3 SOLN	KIO_3 SOLN EQUIV. TO STRYCHNINE IN 20 ML. FILTRATE	CALC'D KIO_3 SOLN EQUIV. TO TOTAL STRYCHNINE	STRYCHNINE FOUND	ERROR
Blank		5.26				
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*
0	15	3.48	1.78	2.23	4.97	− 0.8
1	14	3.47	1.79	2.24	4.99	− 0.4
2	13	3.45	1.81	2.26	5.04	+ 0.6
4	11	3.46	1.80	2.25	5.01	0.
7	8	3.42	1.84	2.30	5.12	+ 2.2
12	3	3.28	1.98	2.48	5.53	+10.4

Concentrated hydrochloric acid not in excess of 1/6 of the total volume does not adversely affect the precipitation. The presence of some free acid appears to be advantageous in hastening the crystallization and settling of the precipitate. The equivalent of 1 cc. of concentrated hydrochloric acid in 25 cc. of the solution is ample for this purpose.

EFFECT OF SUCROSE AND LACTOSE

To determine whether sucrose and lactose, sometimes used as diluents in medicinal tablets, would affect either the precipitation of strychnine by the potassium mercuric iodide solution or the iodate titration of the filtrate the experiments described in Table 3 were made. The results indicate that sucrose and lactose do not adversely affect the determination. Strychnine in hypodermic tablets may therefore be determined without preliminary separation of the alkaloid.

TABLE 3.—*Effect of sucrose and lactose*

		STRYCHNINE TAKEN	20 ML. FILTRATE REQUIRES KIO_3 SOLN	CALC'D KIO_3 SOLN REQUIRED FOR 25 ML. OF FILTRATE	KIO_3 SOLN EQUIV. TO STRYCHNINE	STRYCHNINE FOUND	ERROR
Average blank				6.59*			
	mg.	*mg.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*
Carbohydrate							
Lactose	400	5.01	3.47	4.34	2.25	5.01	0.
Lactose	400	0.	5.28	*6.60*			
Sucrose	400	5.01	3.49	4.36	2.23	4.97	−0.8
Sucrose	400	0.	5.30	*6.63*			
None		5.01	3.45	4.31	2.28	5.08	+1.4
None		0.	5.23	*6.54*			

* Average of three results shown below in italics.

EFFECT OF STARCH

Starch, rendered soluble by treatment with hydrochloric acid does not adversely affect either the precipitation or the titration as is shown by the

TABLE 4.—*Effect of starch*

	STARCH	STRYCHNINE TAKEN	20 ML. FILTRATE REQUIRES KIO_3 SOLN	CALC'D KIO_3 SOLN REQUIRED FOR 25 ML. OF FILTRATE	KIO_3 SOLN EQUIV. TO STRYCHNINE	STRYCHNINE FOUND	ERROR
Average blank				6.59*			
	mg.	*mg.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*
	400	5.01	3.48	4.35	2.24	4.99	−0.4
	400	5.01	3.48	4.35	2.24	4.99	−0.4
	400	None	5.25	*6.56*			
	400	None	5.29	*6.61*			
	None	None	5.27	*6.59*			

* Average of three results shown below in italics.

following data: 0.4 gram of starch was treated with 1 cc. of cold hydrochloric acid (1+1). After solution was effected, 5 ml. of the strychnine solution (containing 5.01 mg. of strychnine) was added, and the mixture was washed into a 25 cc. graduated flask; 3 cc. of the reagent was added, and the determination was completed as described. The results are shown in Table 4.

It was thought that it might be possible to determine the strychnine in compressed tablets without preliminary separation of the alkaloid from the excipients. A few experiments with tablets of unknown excipient composition proved that this procedure is unreliable. Evidently some materials used as excipients affect either the precipitation or the subsequent titration. In specific instances in which it is known that no interfering substances are present the method may be applied directly. In the case of unknown excipients the only safe procedure is to extract the alkaloid.

EFFECT OF NITRATE

Strychnine nitrate is used in medicine. Since the hydrochloric acid used in the iodate titration might conceivably react with the nitric acid and thus vitiate the results, the following experiments were made:

Some molded tablets labeled "1/30 grain Strychnine Nitrate," which contained no starch and which dissolved in water except for a little acid-insoluble material, presumably talc, were counted, weighed, and pulverized. Weighed portions of the powder were treated as follows: The alkaloid was extracted with chloroform in the usual way in tests numbered 1, 2, and 3; to the chloroformic solutions was added a small quantity of hydrochloric acid (1 + 2), and the chloroform was evaporated. In the other six tests the powdered sample was dissolved in water. To each of Nos. 1, 4, and 7 was added 5 mg. of potassium nitrate to accentuate the effect, if any, of nitrate. The strychnine was then determined as described. The results (Table 5) show that nitrate in small amount is without effect

TABLE 5.—*Effect of nitrate*

(Eighty tablets weigh 9.2024 grams. Average weight 115 mg.)

	EXPERIMENT NO.	WT. OF POW'D. TABLETS	ADDED KNO_3	REAGENT ADDED	20 CC. OF FILTRATE REQUIRES KIO_3 SOLN	KIO_3 SOLN EQUIV. TO STRYCHNINE IN 20 CC. FILTRATE	CALC'D KIO_3 SOLN EQUIV. TO TOTAL STRYCHNINE	STRYCHNINE NITRATE TOTAL		STRYCHNINE NITRATE TABLET
		mg.	*mg.*	*cc.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*	*mg.*
		Blank	—	3	5.24					
		Blank	—	5	8.84					
		Blank	—	7	12.38					
	1	1.0194	5	7	6.60	5.78	7.23	19.15	1.879	2.16
Alkaloid	2	1.3141	—	7	5.13	7.25	9.06	23.99	1.826	2.10
shaken	3	0.8900	—	5	3.82	5.02	6.28	16.63	1.869	2.15
out										
	4	0.7617	5	5	4.45	4.39	5.49	14.54	1.909	2.20
Powdered	5	0.6115	—	3	1.90	3.34	4.18	11.07	1.810	2.08
tablets	6	0.6473	—	5	5.07	3.77	4.71	12.47	1.926	2.21
dissolved	7	0.3740	5	3	3.17	2.07	2.59	6.86	1.834	2.11
in water	8	0.4347	—	3	2.76	2.48	3.10	8.21	1.889	2.17
	9	0.3750	—	3.01	3.19	2.07	2.59	6.86	1.829	2.10
								Average		2.14
								Label declaration		2.16
								By N.F. VI*		2.11

* Results obtained in another laboratory.

on the results. In this particular case the excipients were without influence on the strychnine determination.

The maximum deviations from the average in the nine determinations (Table 5) are +3.3 per cent and −2.8 per cent. The N. F. VI determination was reported from another laboratory. The same laboratory reported by an unofficial modification of the N. F. method 2.04 mg. of strychnine nitrate per tablet.

Results of analyses of other tablets are given in Table 6. Sample No. 56091-B, red-coated tablets labeled "Strychnine sulfate 1/60 grain." Average weight, 85.4 mg. Weighed portions of the powdered tablets were treated with 1 cc. of hydrochloric acid (1+1) to dissolve the starch, the solution was diluted and treated with sodium carbonate in excess, and the alkaloid was extracted with chloroform. After the addition of a little dilute hydrochloric acid the chloroform was evaporated and the strychnine in the aqueous solution determined. Deviations from the average of three determinations were −1.8 +0.8, and +1.0 per cent, respectively. Sample No. 20660-B, pink-coated tablets labeled "Strychnine sulfate 1/60 grain." Average weight, 96.7 mg. The excipient contained

TABLE 6.—*Results on strychnine in compressed tablets*

WT. OF POW'D. TABLETS	REAGENT ADDED	20 CC. OF FILTRATE REQUIRES KIO_3 SOLN	KIO_3 SOLN EQUIV. TO STRYCHNINE IN 20 CC. OF FILTRATE	CALC'D KIO_3 SOLN EQUIV. TO TOTAL STRYCHNINE	STRYCHNINE SULFATE TOTAL		STRYCHNINE SULFATE TABLET
gram	*cc.*	*cc.*	*cc.*	*cc.*	*mg.*	*per cent*	*mg.*
			56091-B				
Blank	3	5.30					
0.6943	3	3.37	1.93	2.41	6.88	.991	0.846
0.8562	3	2.86	2.44	3.05	8.71	1.017	0.868
1.0434	4	4.09	2.98	3.72	10.62	1.019	0.870
					Average		0.861
					Label declaration		1.088
					By N.F. VI*		0.875
			20660-B				
Blank	3	5.33					
0.2002	3	4.68	0.65	0.81	2.313	1.155	1.117
0.6756	3	3.25	2.08	2.60	7.423	1.099	1.063
				Average			1.090
				Label declaration			1.088
				By N.F. VI*		1.08	1.044
						1.10	1.064

* Results obtained in another laboratory.

some ingredient, which rendered it difficult to wet with aqueous liquids. Deviation from the average, +2.5 per cent.

SUMMARY

Strychnine in amounts as low as 5 mg. can be determined by precipitation with a standard potassium mercuric iodide solution and titration of the excess iodide with standard potassium iodate.

ANALYSIS OF ZINC PHENOLSULFONATE*

By MILDRED W. EVANS

The method in the United States Pharmacopoeia IX, p. 492, and now in National Formulary VI, p. 435, for the analysis of zinc phenolsulfonate is admittedly unsatisfactory, allowing a variation in results from 99.5 to 104.5 per cent. The disadvantages of this method have been summarized by Ewe and Gloor[1] who, in turn, suggest a ferrocyanide method with external indicator. This method is no doubt an improvement over the sulfide method, but it suffers the usual inaccuracies of an indirect method.

Grant,[2] on the other hand, maintains that the bromide-bromate method

* Contribution from the Chemistry Laboratory of Wheaton College, Norton, Mass.

[1] *J. Am. Pharm. Assoc.*, **10**, 101 (1921).

[2] *This Journal*, **14**, 351–5 (1931).

for phenols in United States Pharmacopoeia IX will yield results to within one per cent of the true value if catalysts are absent and if the excess of bromine is within 2–5 cc. of 0.1 *N* solution. Harris[1] and Grant[2] have shown that the proper choice of concentrations of reactants and of reaction time makes the bromide-bromate method possible although not really satisfactory. A direct titration method suggested by Grant[2] is, according to the writer, only "fairly" satisfactory since it is tedious and requires practice. The writer also found that the presence of 0.5 gram of mercuric chloride caused a one per cent decrease, and the presence of 5 grams of potassium iodide caused a one per cent increase in the result.

The present investigation was undertaken to determine the possible usefulness of an iodometric method of analysis, similar to the one used by Evans[3] for sodium salicylate and by Gesell and Evans[4] for acetylsalicylic acid.

A specimen of zinc phenolsulfonate was prepared by the method of Takagi and Kutani[5] and was recrystallized many times from hot water. The sample was then analyzed by the following method, in which the reaction involved is expressed by the equation:

$$[C_6H_4(OH)SO_3]_2Zn + 4I_2 \rightarrow [C_6H_2(OH)I_2SO_3]_2Zn + 4HI.$$

METHOD

From 1.5 to 2.0 grams of zinc phenolsulfonate was weighed in a closed container, dissolved in water, transferred to a 250 cc. volumetric flask, and made up to volume, and 25 cc. of this solution was pipetted into a glass-stoppered iodine flask. 45 cc. of an approximately 0.1 *N* Na_2CO_3 solution was added, the solution was heated for a few minutes to hasten the later reaction with iodine, and 35 cc. of standardized 0.1 N iodine solution was added slowly from a buret, the flask being shaken constantly. The flask with its contents was heated over a 1½ inch Bunsen flame for about 15 minutes with the stopper kept wet with KI solution to prevent any escape of I vapors. After the flask had stood for 10 minutes, the contents were cooled to room temperature by holding the flask under the water tap and acidified with H_2SO_4 (1+1). The excess I was titrated with standardized 0.1 *N* $Na_2S_2O_3$ solution. 1 cc. of 0.1 *N* I = 0.0051465 gram of anhydrous zinc phenolsulfonate or to 0.0069465 gram of crystalline zinc phenolsulfonate.

Since zinc phenolsulfonate loses some of its water of crystallization on standing, results of analyses will mean little unless this is taken into account. This may be done by determining the water content of the salt, or by heating the sample until all the water of crystallization has been driven off and making the analysis on the anhydrous salt. Table 3 shows that this is necessary since here a commercial sample, which was only 98.5 per cent pure, analyzed as 100.9 per cent zinc phenolsulfonate when no account of the water content was taken.

The results of the analysis of two separately prepared samples of zinc phenolsulfonate are recorded in Table 1.

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[1] This Journal., 13, 364–6 (1930).
[2] *Ibid.*, 15, 424–6 (1932).
[3] *J. Am. Pharm. Assoc.*, 12, 225–227 (1923).
[4] *Ibid.*, 228.
[5] *J. Pharm. Soc. Japan*, No. 505, 155–61 (1924).

TABLE 1.—*Results of analysis of two samples of zinc phenolsulfonate*

0.1 N Na_2CO_3	I_2	$Na_2S_2O_3$	0.1 N I_2 USED	
cc.	*cc.*	*cc.*	*cc.*	*per cent*
		Sample 1		
	Weight of Sample		1.2832 grams	
	Factor of Iodine, 0.1 N		0.1297	
	Factor of Thiosulfate, 0.1 N		0.1097	
45	26.00	13.92	18.45	99.9
35	20.00	6.82	18.46	100.0
35	20.00	6.83	18.45	99.9
35	20.00	6.83	18.45	99.9
35	20.00	6.84	18.44	99.8
35	20.01	6.84	18.45	99.9
		Sample 2		
	Weight of Sample		1.4921 grams	
35	20.00	4.10	21.44	99.9
35	20.00	4.10	21.44	99.9
35	20.50	4.72	21.41	99.7
35	20.00	4.09	21.45	99.9

The sodium thiosulfate solution was standardized against potassium dichromate and resublimed iodine.

A sample of crystallized zinc phenolsulfonate was procured from a commercial laboratory and analyzed, with the results recorded in Table 2.

TABLE 2.—*Results on crystallized commercial sample*

Factor of Iodine, 0.1 N 0.1120
Factor of Thiosulfate, 0.1097

WEIGHT	TREATMENT OF SAMPLE	Na_2CO_3	I_2	$Na_2S_2O_3$	0.1 N I_2 USED	
grams		*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
1.5200	As was	45	30.00	10.67	21.90	100.1
		45	30.00	10.65	21.92	100.2
1.2354	Heated to	45	30.00	8.86	23.88	99.6
	125° C.	45	30.00	8.86	23.88	99.6
1.7499	Humidor	45	30 00	7.64	25.23	100.2
	1½ Hrs.	45	30.00	7.66	25.20	100.0

A second commercial sample assayed with the results recorded in Table 3.

The sodium thiosulfate solution used for these analyses was standardized against Bureau of Standards sodium oxalate and resublimed iodine.

SUMMARY

An iodometric method of analysis for a phenolsulfonate has been developed. This method gives results that check to 0.2 per cent. It allows

TABLE 3.—*Results on commercial sample No. 2**

WEIGHT	TREATMENT OF SAMPLE	Na_2CO_3	I_2	FACTOR	$Na_2S_2O_3$	FACTOR	0.1 N I_2 USED	
grams		*cc.*	*cc.*		*cc.*		*cc.*	*per cent*
1.4024	As was	30	24.86	0.1000	4.49	0.1000	20.37	100.9
		25	24.76	0.1000	4.40	0.1000	20.36	100.9
1.4229	Dried at	37.5	33.00	0.09944	5.33	0.1048	27.24	98.5
	125° C.	30	33.00	0.09944	5.33	0.1048	27.24	98.5
		30	33.00	0.09902	5.18	0.1048	27.25	98.6
1.5013	Dissolved in	40	33.00	0.09877	3.43	0.1048	29.00	99.4
	alcohol	45	34.90	0.09877	5.20	0.1048	29.02	99.5
	P'p'ted. with	50	42.00	0.09877	11.84	0.1048	29.08	99.6
	ether. Dried	45	35.00	0.09845	5.17	0.1048	29.04	99.6
	at 125° C.	50	42.00	0.09845	11.75	0.1048	29.04	99.6

* Analyses made in the laboratories of Lehn and Fink Inc., where this work was started in 1923.

rather wide limits in details of procedure, 10 cc. variance in amount of sodium carbonate solution, and at least 15 cc. in excess iodine. The solution must be heated at least 10 minutes after addition of the iodine.

ESTIMATION OF WORM AND INSECT FRAGMENTS IN TOMATO PRODUCTS

By C. D. WILDER and M. A. JOSLYN (Fruit Products Division, University of California, Berkeley, Calif.)

The procedure used at present for the determination of worm and insect fragments in tomato products is essentially that described by B. J. Howard[1] and Wm. H. Harrison.[2] In this method a measured volume, usually 200 cc., of the tomato product, is mixed thoroughly with a small volume of gasoline and then diluted with water. The gasoline rises to the surface, carrying with it the worm and insect fragments and at the same time separating them from most of the pulp present. The gasoline, together with a few cc. of the underlying aqueous mixture, is poured through a small filter paper and the number of insect fragments is determined by examination under a wide-field, low-powered, binocular microscope. However, the separation of the pulp is rarely complete, particularly when stable emulsions of gasoline are formed, and the preparation and counting of the plate is rather tedious and time consuming. Therefore a critical study was made of the method, some of the results of which are given in this paper, together with a suggested alternative procedure.

MECHANISM OF SEPARATION

As there is little information on the mechanism of the separation of worm fragments by the gasoline flotation procedure, the following pre-

[1] *Food Industries*, 7, 321 (1935).
[2] *Canner*, 84, 9–10, 18 (1937).

liminary tests were made. Although the composition of the insect fragments has not been completely investigated, it is known that chitin occurs in the outer non-cellular layer or cuticula.[1] The presence of fat-soluble material has not been reported.

An examination of gasoline extracts prepared from tomato sauce indicated that the majority of worm fragments were present at the gasoline-water interface. No fragments were found in the water layer although some were found at the surface of the gasoline. These, however, were always small setae. It is possible that they might have been buoyed up by the air bubbles. The worm fragments were found not to be wet by water although they were readily wet by gasoline, which was found to spread quickly over the surface.

Several tests were made to determine whether the insect fragments were concentrated at the interface by air bubble flotation process. It was found that thorough vacuumization of gasoline extracts, to remove all air bubbles that might serve to buoy up the fragments, had no effect upon the distribution or number of fragments. Furthermore, fragments added to gasoline layered over water were found to settle to the interfacial layer and after agitation to settle to the bottom of the meniscus. None of the fragments, even after agitation, was found to move either into the gasoline or into the water.

These and other similar observations indicate that the first stage in the process is the wetting of the fragment by the gasoline. The violent churning of the gasoline and the tomato product, by breaking up the gasoline into small droplets and dispersing these uniformly throughout the medium, insures the selective wetting of each fragment. The subsequent addition of warm water tends to separate insect fragments from the pulp particles that may be attached to them and thus allows the fragments to rise through the liquid to the gasoline-water interface. The mass motion of the air incorporated in the mixture by churning aids this process. On reaching the surface of the water the drops of gasoline, with the attached worm fragments, coalesce, forming the gasoline layer, and the fragments drop to the bottom of this layer. It was observed that the fragments slip around water or air dispersed in the gasoline layer and sink to the lowest possible point of this layer.

In this process the separation of the fragments from the particles of the pulp is rarely complete. When an emulsion of gasoline that is difficult to break forms in the first extraction, the plate obtained is usually dirty and difficult to examine. A clean plate with a minimum of pulp present requires from 10 to 20 minutes for examination, while a plate that has a large amount of pulp present may require an hour or more.

Some products form a stable gasoline emulsion that is very difficult

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[1] Wigglesworth, Insect Physiology, pp. 1–4. Methuen & Co., London; Packard, A Textbook of Entomology, pp. 28–30. The Macmillan Co., New York.

to break. These products usually contain more gasoline-soluble material that may function as an emulsifying agent.

TESTS WITH OTHER FAT SOLVENTS

It was thought that a better separation might be obtained by the use of solvents not miscible with water other than gasoline. The results obtained with these solvents are given in Table 1. C. H. McCharles of the California State Food and Drug Laboratory[1] tested numerous solvents including various petroleum distillate fractions, but found none superior to gasoline.

EFFECT OF SEVERAL OXIDIZING AGENTS

The next step was to try various treatments for the purpose of destroying the natural coloring matter and emulsifying agents. The results with tomato sauce are shown in Table 2. The acidic permanganate, appearing to be the most promising, was then applied to the official procedure.

To a 2 liter flask fitted with a rubber stopper plunger was added 200 cc. of tomato sauce and 35 cc. of gasoline. This mixture was churned well, 200 cc. of 12 *N* sulfuric acid was added, and the solution was mixed again. Then 200 cc. of a permanganate solution (100 grams of potassium permanganate per liter of water) was added, and after the reaction was complete the flask was filled with water and allowed to stand. The gasoline layer was removed, washed again, and filtered. No emulsion was found in the gasoline layer and the pulp present was colorless and very finely divided. The fragment count agreed with that of an untreated sample.

TABLE 1.—*Results obtained with solvents not miscible with water*

SOLVENT	CONDITION OF PLATE	TOTAL NUMBER OF FRAGMENTS
Ethyl acetate	Due to evaporation no results were obtained	—
Xylene	The plate was poor. Large amount of coloring matter and pulp present	58
Toluene	Cleaner than above. Less coloring matter and skin present	34
Gasoline	Cleanest plate. Some coloring matter and skin present	54

Fragments of common house flies were boiled in acid and alkali, washed well, and then added to the acidic permanganate. After a period of 15 minutes the fragments showed no changes. The fine transparent structure of the wing was undamaged. These tests and other similar ones indicated that the permanganate had no injurious effect upon the worm and insect fragments.

[1] Personal communication.

The next step was the substitution of a one liter separatory funnel for the flask. This funnel was used in all other determinations listed under the potassium permanganate method. When the sauce was treated with the acidic permanganate the gasoline separated satisfactorily, floated in a clear layer, and contained very little pulp. A violent evolution of gas with foaming occurs after the addition of the permanganate, hence it was found best to add it in small portions (50 cc.).

TABLE 2.—*Action of oxidizing reagents upon a sample of tomato sauce*

REAGENT	REACTION
Hydrogen peroxide	A slow evolution of gas but no other signs of reaction. May have been catalytic decomposition. No decolorization.
Bleaching solution Calcium hypochlorite	Slow evolution of gas and a slight decolorization after several hours.
Potassium chromate and sulfuric acid	A slow reaction occurred in which a decrease in color was found. With large excess complete decolorization did not occur.
Potassium permanganate and sulfuric acid	Very rapid reaction, accompanied by evolution of gas, and resulting in complete decolorization.

Attempts were made to find the amount of permanganate that was required to give the cleanest plate. No additional benefit was noted after the pulp had become pink in color. The separation of the gasoline was easily accomplished by drawing off the aqueous liquid at the bottom. The remaining gasoline was washed with water to remove the pulp that was found at the interface. (The washing is easily done and can be quickly repeated as many times as necessary.) The cleanliness of the remaining gasoline makes filtering easy.

The preliminary success of the potassium permanganate treatment next led to tests comparing it with the official procedure. Samples of different products and of the same product from different factories were taken to determine whether variations in machinery or varieties of tomatoes used would have any effect. The results found are given in Table 3. Table 4 gives the results of a series of tests upon the same sample of tomato paste.

The counts show a fairly close agreement, although the pastes give some wide variations. No definite tendency for either method to give high results was evident.

Comparison of the plates obtained shows in each case that the plate resulting from the use of potassium permanganate is freer of coloring matter, gummy substances, and pulp. In Fig. 1, 1 and 2, the plates on the left were prepared by the official procedure and those on the right (the same sample) were prepared by the potassium permanganate procedure. This pulp was prepared at the factory by the use of a large finishing machine with fairly coarse screen, which accounts for the large pieces

of pulp seen in the plates. A large amount of gummy and gelatinous material was present on the plates that were prepared by the official procedure. The other plates did not contain this material, and even with some pulp present they were easily examined. While the plates shown in Fig. 1 were the dirtiest ones found, the others exhibited similar marked differences.

TABLE 3.—*Comparison of the official and the $KMnO_4$ procedures*

SAMPLE NUMBER	TYPE	WORMS AND INSECT FRAGMENTS— OFFICIAL METHOD	$KMnO_4$ METHOD
1	paste	38	36
2	paste	40	44
3	sauce	8	8
4	sauce	8	10
5	sauce	16	13
6	sauce	13	11
7	sauce	9	11
8	sauce	17	17
		18	19
			23
9	sauce	16	15
		18	19
		19	20

TABLE 4.—*Number of worm and insect fragments determined by the official and the $KMnO_4$ methods by repeated counts on a sample of tomato paste*
(Each value given represents a separate amount of paste)

OFFICIAL METHOD		$KMnO_4$ METHOD	
41	45	36	45
45	44	45	47
44	56	56	40
58	46	44	52
47	41	41	54
			50
			42

Average: Official 46.7; $KMnO_4$ 46.0

Comparison of the time required is of interest. For the official procedure the time of preparation of one sample is from 20 to 30 minutes, whereas with the potassium permanganate procedure the time required is from 5 to 10 minutes. This saving is further increased because of the shorter time required for examination.

The sample (Fig. 1) illustrates the difficulties that will be encountered with samples that are not run through a fine finishing machine, because of the excessive amount of pulp present. This pulp was peculiar in behavior, in that it resisted the decolorizing action of the permanga-

nate, even large excesses having no effect. The pulp rose so rapidly that it could not be removed completely by washing. The same type of pieces was found in the plate obtained by the official procedure.

To remove this residual material, the permanganate-treated material obtained from a sample of tomato sauce that was found to contain a large amount of skin was subjected to several cellulose solvents, with the negative results shown in Table 5. Lead acetate and other salts were also used, but were not successful. Changes in the method of addition were tried but very little improvement was found.

TABLE 5.—*Action of cellulose solvents upon tomato pulp after treatment with acidic permanganate*

SOLVENT	REACTION
Schweizers reagent	Dissolved the colorless pulp but not the colored residue
Concentrated H_3PO_4	Dissolved the colorless pulp but not the colored residue
Zinc chloride and hydrochloric acid	No action
Nitric acid	No action

The second difficulty encountered was the presence of a colorless air-water emulsion at the interface. Filtration did not destroy it, as it remained upon the filter paper, and repeated washing with hot or cold water had no effect. In all other respects the plate was clean; all coloring matter and coarse pieces of pulp were absent. Varying the order of addition of acid, gasoline, and potassium permanganate was next tried. It was found that when the gasoline was added first and mixed well, next the acid, and finally the permanganate, there was a minimum of foam present. The counts obtained are given in Table 6. They indicate that the variation in sequence of addition of reagents had no effect upon the number of worm fragments found.

TABLE 6.—*Number of fragments found when the sequence of operations was varied*

PROCEDURE	NUMBER OF FRAGMENTS
Adding gasoline and acid before mixing	11—11
Adding gasoline, mixing, and then decolorizing	10—12

The amount of gasoline lost during the process was determined by measuring the amount recovered after filtration. When 35 cc. of gasoline was used, the measurements gave a recovery of 19–20–20 cc. Examination of the discarded fluid showed only a few drops present. The only explanation of this loss is evaporation, solubility of the gasoline in water, or some reaction with the permanganate.

Attempts were made to vary the amount of gasoline used and to determine the effect upon the count. The results are given in Table 7.

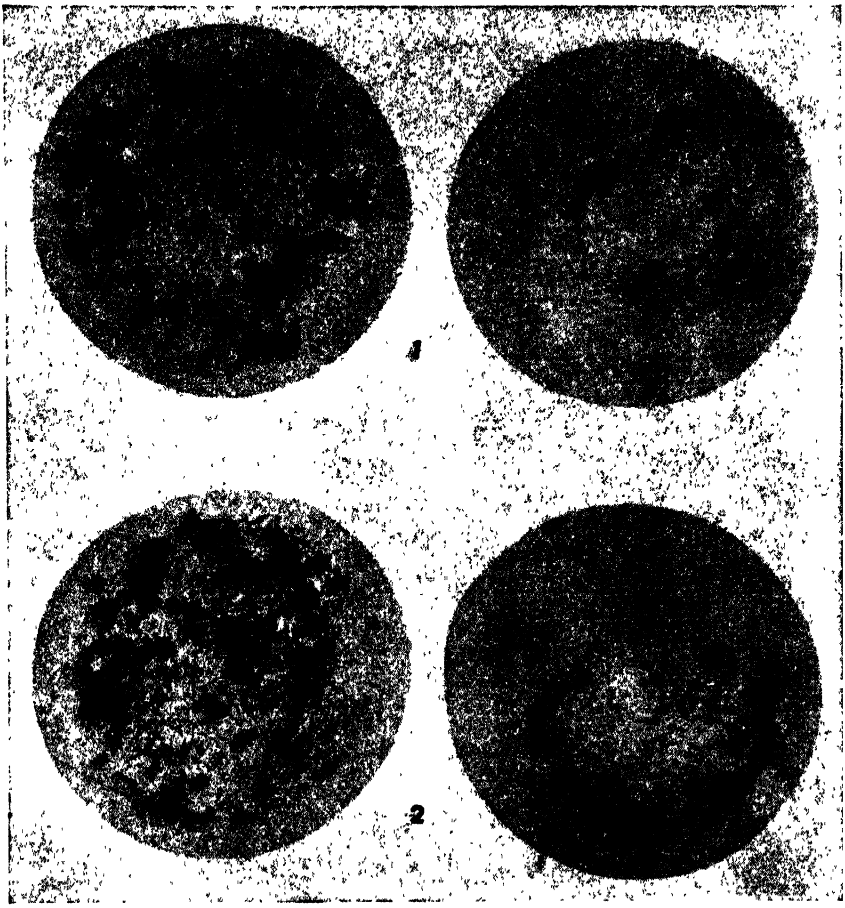

FIG. 1.—APPEARANCE OF PLATES PREPARED FROM SAMPLE BY THE USUAL AND BY THE POTASSIUM PERMANGANATE PROCEDURES. PLATES AT RIGHT PREPARED BY POTASSIUM PERMANGANATE PROCEDURE, THOSE AT LEFT BY USUAL PROCEDURE.

The results of this experimental work has led to the suggesting of the following method for the preparation of samples of tomato products to determine the number of worm and insect fragments present.

METHOD

REAGENTS

Sulfuric acid.—12 *N.*

Potassium permanganate solution.—Dissolve 100 grams of $KMnO_4$ in 1 liter of warm water and filter.

APPARATUS

Separatory funnel.—1 liter.

Graduated cylinder.—250 cc.

Graduated cylinder.—50 cc.
Filter paper.—7 cm.
Petri dish.—10 cm. diameter.

PROCEDURE

Put 200 cc. of the tomato sauce, juice, or catsup (or 100 cc. of tomato paste) into the separatory funnel. Add 35 cc. of gasoline, stopper, invert, and shake. Open the stopcock slowly to release the pressure. Close the stopcock and shake well until the gasoline is thoroughly emulsified. Open the stopcock to release pressure, close, and invert. Add 200 cc. of the sulfuric acid solution. Move in a rotary motion until the gasoline is well mixed.

TABLE 7.—*Effect on the total number of fragments found of varying the amount of gasoline* (modified procedure)

CC. OF GASOLINE	NUMBER OF FRAGMENTS
25	50—42—46
35	54—59
50	53—57—55

Add 150 cc. of the permanganate solution in 50 cc. portions to prevent excessive foaming. After the addition of each portion move the funnel vigorously in a rotary motion until all traces of the permanganate and the manganese dioxide have disappeared. After the last addition, fill the funnel with water and allow to stand for 1 minute.

Drain off the lower layer of water and pulp until the bottom of the gasoline layer is about 2 inches from the stopcock. Add 200 cc. of water, allow to stand for 20–30 seconds, and then drain as before. Add 100 cc. of water and drain again as soon as the gasoline has risen to the surface. If all pulp is not gone, repeat the washing until the water layer is free of pulp.

When the washing is completed, drain off the water until the bottom of the gasoline layer is about one-half inch from the stopcock. Filter the remaining water and gasoline through the filter paper, washing the inside of the funnel well with water. Examine the filter paper under the microscope for worm and insect fragments in the usual manner, as described by Howard.

BOOK REVIEW

Qualitative Analysis and Chemical Equilibrium. By T. R. Hogness, Associate Professor of Physical Chemistry at University of Chicago, and Warren C. Johnson, Associate Professor of Chemistry, University of Chicago. xii+417 pp. Henry Holt and Co., New York. 1937. Price, $2.75.

This textbook in qualitative analyses is divided into two sections. The first section is given over to such fundamental considerations as properties of solids, liquids, and solutions; reaction velocity and equilibrium in homogeneous and heterogeneous systems; solubility product; hydrolysis; complex ions, oxidation and reduction, etc. The second section treats of group separations and tests for individual elements. The authors make it plain in the preface, however, that this separation is somewhat artificial, and that "theory and practice are coordinated at every opportunity."

The theoretical discussions are clear and readily understandable. If any one topic should be singled out as particularly well done, it would probably be the discussion of the law of mass action. Instead of the more or less stereotyped matheematical presentation commonly found in textbooks, the authors make use of a graphical treatment whereby the student can form a mental picture of "the quantitative statement relating the velocity of a reaction to the concentrations of its reactants."

The chapter on oxidation and reduction (33 pages) is complete and well presented. A table of 111 oxidation-reduction half-reactions is given, together with several examples to illustrate the use of the table.

Each chapter is concluded with a number of questions and problems, a commendable feature. The appendix (67 pages) contains data ordinarily found in chemists' handbooks, such as the use of logarithms and exponential numbers; use of the slide rule; *p*H values; ionization constants of weak acids and bases; a table (3 pages) of solubility-product constants; a table (31 pages) giving "physical properties of compounds of ions of the analytical groups," and finally a table of 4-place logarithms.

A student mastering the contents of this book will have a good foundation, not only in qualitative analysis but in theoretical chemistry as well. These two subjects should be correlated, and they are in this book.

It might have contributed to the completeness of the theoretical discussions if brief mention had been made of the historical development of some of the subjects. Electronic structures are considered, but the fact that Lewis and Langmuir originated the ideas is not mentioned. Similar comment might be made with regard to the law of mass action, colloids, and catalysis.

On the whole Qualitative Analysis and Chemical Equilibrium is an excellent textbook, and it is highly recommended to instructors in this subject.—O. A. Nelson.

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